



Correlation of Lumbar Intervertebral Disc Degeneration, Abdominal Aortic Calcification on Plain X-ray and Pineal Gland Calcification at CT in Patients with Low Back Pain

Mst. Maksuda Khatun^{1*}, Mohammad Shahin Akhter², Abul Hasan³, Salma Shahnawaz Parvin⁴, Md. Towrit Reza⁵

¹Assistant Professor, Department of Radiology and imaging, Bangabandhu Sheikh Mujib Medical College (BSMMC), Faridpur, Bangladesh,

Email: shahinjodder75@gmail.com, Orcid ID: 0000-0002-2689-831X

²Assistant Professor, Department of Orthopedics, Bangabandhu Sheikh Mujib Medical College (BSMMC), Faridpur, Bangladesh,

Email: shahinjodder75@gmail.com, Orcid ID: 0000-0003-1749-8821

³Registrar, Department of Orthopedics, Bangabandhu Sheikh Mujib Medical College Hospital (BSMMCH), Faridpur, Bangladesh, Email: hasandrmsortho@gmail.com Orcid ID: 0000-0002-4217-5656

⁴Assistant Professor, Department of Radiology and imaging, Bangabandhu Sheikh Mujib Medical College (BSMMC), Faridpur, Bangladesh,

Email: dr.salma.mdgmail.com, Orcid ID: 0000-0002-2689-831X

⁵Assistant Professor, Department of Radiology and imaging, Bangabandhu Sheikh Mujib Medical College (BSMMC), Faridpur, Bangladesh,

Email: towritrera@gmail.com, Orcid ID: 0000-0002-2689-831X

*Corresponding author

Received: 22 February 2022

Revised: 21 April 2022

Accepted: 30 April 2022

Published: 23 June 2022

Abstract

Background: The lumbar spine, or low back, is a remarkably well-engineered structure of interconnecting bones, joints, nerves, ligaments, and muscles all working together to provide support, strength, and flexibility. However, this complex structure also leads the low back susceptible to injury and pain. To find out the correlations among plain radiographic findings of lumbar intervertebral disc degeneration, abdominal aortic calcification & CT findings of pineal gland calcification in low back pain subjects. **Material & Methods:** This observational analytical study was carried out in the Department of Radiology and imaging of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) from 1 January 2011 to 31 December 2012. A total of 100 subjects attending the department of Radiology and imaging, BIRDEM for X-ray of the Lumbosacral spine and CT scan of the brain with low back pain were enrolled first for the study. A complete history was taken either from the patient or accompanying attendants. Relevant investigations reports were collected. All the information was recorded in the data collection sheet. Collected data were classified, edited, coded and entered into the computer for statistical analysis by using SPSS-23. **Results:** The mean age of study subjects was 61.26 years with a standard deviation of the mean (SD) of 13.34 years and their age ranged from 41 to 74 years. It was observed that nearly two-thirds (61.7%) of the subjects were male and 38.3% were female and the male-female ratio was 1.6:1. It was seen that majority of the subjects had a density of the Pineal gland ranging from +51 to +150 HU. Only 4 subjects had a density of Pineal gland ranging from +351 to +1000 HU. Meant SD density of the Pineal gland among a total of 30 subjects was 136.98164.11 HU. In Group X, the density of Pineal Gland was 83,57 14.45 HU. The density of the Pineal gland was 134.65±13.23HU and 151.66±21.32 HU in Group Y and Group Z respectively. Some parameters of the degenerative disc disease and aortic wall calcification. had a significant positive association with calcification. with the density of Pineal gland calcification. **Conclusions:** The study was undertaken to find out the Correlation between lumbar intervertebral disc degeneration, abdominal aortic calcification on plain X-ray and Pineal gland calcification at CT in low back pain subjects. The data obtained showed that the density of pineal gland calcification is statistically significant with increasing age. There was also a positive association between intradiscal calcification and density of pineal gland calcification, but no significant association among other parameters with the density of pineal gland calcification.



Keywords:- Plain X-Ray, Pineal Gland

INTRODUCTION

Low back pain, one of the most common causes of disability for the working population, can be induced by the degenerative collapse of the disc tissue as degenerative disc disease (DDD) and it represents a major concern for spinal surgeons & neuroradiologists.^[1,2] Over many years, the understanding of DDD has received much attention because of its clinical importance. About 60-80% of adults suffer from low back pain in their lives.^[3] Degenerative diseases of the spine are divided according to the site involved. Degeneration at the nucleus pulposus leads to disc degeneration, at the annulus fibrosus to spondylosis deformans and apophyseal and costovertebral joints to osteoarthritis. Degenerative disc disease may occur anywhere in the spine but is most common in the lower cervical and lower lumbar spine.^[4] The most common location of these changes is the lumbar spine.^[5] The characteristic findings of discogenic degenerative change on conventional radiographs include loss of disc height, irregularity and sclerosis of the endplates. One of the dominant features is the formation of new bone including osteophytes. On conventional radiography, degenerative change of the facet joints can present as increased sclerosis and oblique projections, joint spaces narrowing can often be defined. Plain X-ray is very popular for diagnosis of low back pain as well as assessment of the degenerative spine. In our country, the pattern of day to day living, nonscientific vehicles and roads, occupational hazards the incidence of low back pain and degenerative changes in the spine are

gradually increasing.^[6] A radiologist can play an important role by properly interpreting the plain radiograph to guide the clinician in diagnosing and managing degenerative changes in the lumbosacral spine. In another study, the three radiographic parameters height-loss, osteophytes and intradiscal calcifications correlate significantly with the morphological degree of degeneration.^[7] At present, it has been well established that DDD is associated with progressive changes in the collagenous matrix composition and morphological features of the disc tissue.^[8] Despite the recent advances in MRI, plain radiography is still an important method for the evaluation of these changes in subjects and low back pain. The percentage of individuals with the degenerating disc increases with age, suggesting a relationship between intervertebral disc degeneration and advancing age.^[8] Numerous studies have also confirmed that atherosclerotic lesions increased with age. Although it has been suggested that insufficient blood supply in the feeding arteries of the lumbar spine owing to atherosclerotic in the abdominal aorta may be a causative factor in disc degenerative changes in the intervertebral disc tissue.^[9,10] In recent years, on the other hand, much interest has been focused on the pineal gland & its neurohormone melatonin (MEL).^[11] Radiologically, it has long been documented that the incidence of calcification of the pineal gland increases with age.^[12,13] As a result, circulating MEL decreases with age, as the increasing degree of pineal calcification might indicate a decrease in MEL production.^[14] Importantly, it has been reported that ageing is

associated with an increased incidence of cancer, and infectious & degenerative diseases, possibly due to diminished MEL production.^[15] At present, data relating to MEL deferring some age-related degenerative conditions, as well as those on the relationship between the pineal gland & ageing, is accumulating rapidly. To the best of our knowledge, there is no such study investigating the effects of pineal calcification as a sign of MEL deficiency upon degenerative changes and systemic atherosclerosis in our country. In this study, it was tried to find out the effect of pineal calcification, which reflects the deficient secretory activity of the gland, may exert a role in the onset and pathogenesis of intervertebral disc degeneration and/or systemic atherosclerosis.

MATERIAL AND METHODS

This observational analytical study was carried out in the Department of Radiology and imaging of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) from 1 January 2011 to 31 December 2012. A total of 100 subjects attending the department of Radiology and imaging, BIRDEM for X-ray of the Lumbosacral spine and CT scan of the brain with low back pain were enrolled first for the study. Purposive sampling (nonrandom sampling) was followed considering the inclusion and exclusion criteria.

Inclusion criteria:

- Adult patients.
- Patients with low back pain referred to the dept. of Radiology and Imaging for X-ray of their lumbar spine and CT scan of the brain for different clinical symptoms (Headache, Memory loss, TIA etc).

Exclusion criteria:

- Patients suffering from neoplastic diseases.
- Patients with H/O trauma in L/S spine and previous lumbosacral spinal surgery. with known infective spondylitis, congenital anomaly or
- Patient's systemic diseases involving the spine.
- Stroke patients.

All the relevant collected data were compiled on a master chart first, then organized by using a scientific calculator and standard statistical formula. Percentages were calculated to find out the proportion of the findings. Further statistical analysis of the results was done by computer software devised as the statistical package for the social sciences (SPSS). The results were presented in tables, figures and diagrams. Variables were demonstrated as (Mean±SD) for the parameters. The statistical analysis of the results among the groups was carried out by using the chi-square test and one-way analysis of variance (ANOVA). The Chi-square test was used to evaluate the association among different parameters. A probability value <0.05 was considered significant.

RESULTS

A total of 60 adult patients having low back pain were included in this study. These patients were referred to the Department of Radiology and Imaging. BIRDEM (Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic disorder) for plain X-ray of the lumbosacral spine and non-contrast CT scan of the brain for different clinical symptoms (Headache Memory loss, TIA etc.) from 1 January 2011 to 31" December 2012. The

age of the patients ranged from 41 to 74 years. All the patients underwent a plain X-ray of the lumbosacral spine and a non-contrast CT scan of the brain. Among 60 subjects, calcification of the pineal gland showed in 30 subjects. So, the density of pineal gland calcification was measured in 30 subjects. All findings of X-ray of the lumbosacral spine and non-contrast CT scan

of the brain were collected in a pre-designed data collection sheet. The plain radiographic findings of lumbar intervertebral disc degeneration and abdominal aortic calcification were compared with pineal gland calcification at non-contrast CT scans of the brain in patients with low back pain.

Table 1: Age distribution of the study subject (n=60)

Age (in a year)	Frequency	Percentage
41-50	15	25.00
51-60	21	35.00
61-70	14	23.33
>70	10	16.67
Mean±SD	61.26±13.34	
Range (min-max)	(41-74) years	

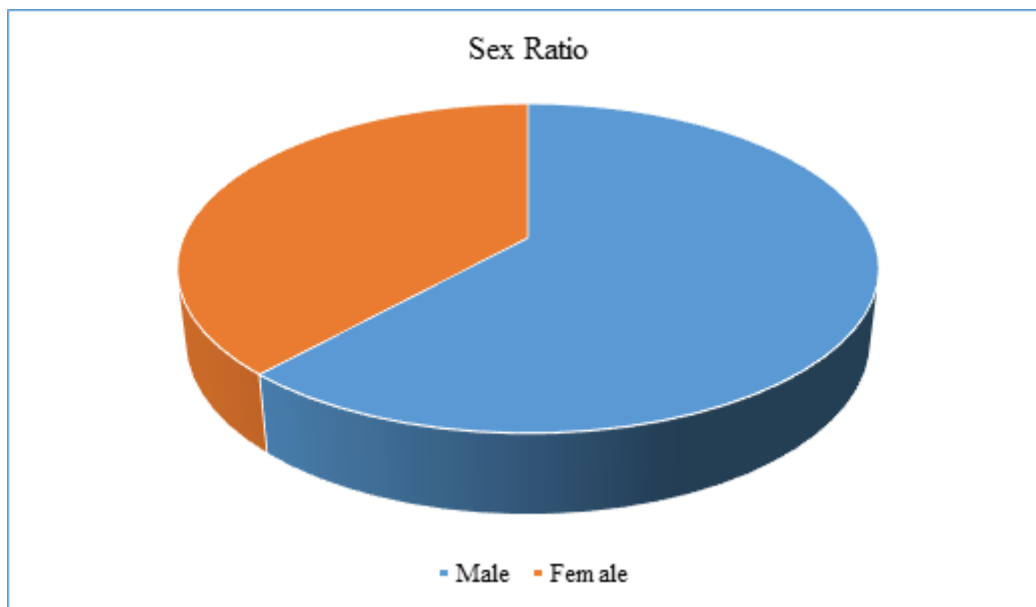


Figure 1: Pie diagram showing the sex distribution of the study subjects.

Table 2: Demographic characteristics of the study subjects (n=60)

Age (Years)	Male (n=37)		Female (n=23)		Mean±SD (in a year)
	N	%	N	%	
Group X (≤ 50) (n=15)	10	27.03	5	21.74	44.44±4.14
Group Y (51-70) (n=35)	20	54.05	15	65.22	61.01±10.87
Group Z (≥ 71) (n=10)	7	18.92	3	13.04	72.84±1.89

Mean±SD (among total)	61.26±13.34
Maximum-Minimum	41-74

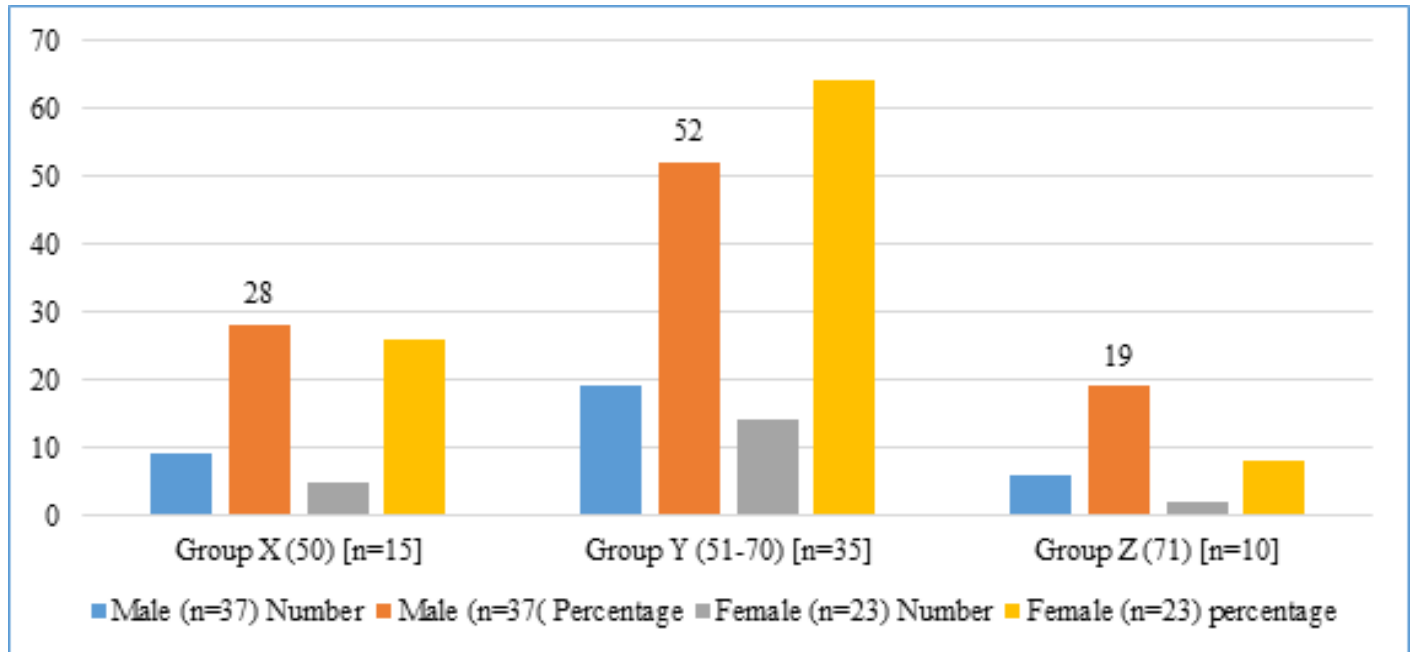


Figure 2: Bar diagram showing demographic characteristics of the study subjects.

Table 3: Disc height loss in group X, Y, Z subjects (n=60)

Disc height loss in percentage	Score	Age group (Years) (Number of the subject)			Statistical test result (t- value/p-value)		
		Group X	Group Y	Group Z	χ ² -value/P-value	ANOVA-value/P-value	r- value/P-value
0-10	0 (n=5)	4	1	0	2.34/0.039	17.83/0.01	0.732/0.01
11-20	1 (n=17)	7	9	1	8.41/0.021		
21-30	2 (n=27)	3	20	4	7.95/0.0011		
31-and above	3 (n=11)	0	6	5	3.89/0.019		

P-value was considered significant when it was <0.05.

Table 4: Posterior osteophyte in group X, Y, and Z subject (N=60)

Type of posterior osteophyte	Score	Age group (Years) (Number of subjects)			Statistical test result (t- value/p-value)	
		Group X	Group Y	Group Z	χ ² -value/P-value	ANOVA-value/P-value
Rounded	0 (n=10)	6	4	0	5.74/0.028	11.23/0.021
Pointed	1 (n=20)	7	13	0	6.99/0.034	
<2mm	2 (n=17)	1	13	3	2.12/0.01	
>2mm	3 (n=13)	0	6	7	5.23/0.043	

P-value was considered significant when it was <0.05.

Table 5: Intradiscal calcification in group X, Y and Z subjects (n=60)

Intradiscal calcification	Score	Age group (Years) (Number of the subject)			Statistical test result (t- value/p-value)
		Group X	Group Y	Group Z	λ-value/P-value
No	0 (n=18)	10	8	0	7.35/0.017
Rim	1 (n=22)	5	17	0	9.63/0.036
Intranuclear (<2mm)	2 (n=13)	0	9	4	4.46/0.042
Intranuclear (>2mm)	3 (n=7)	0	1	6	2.11/0.038

P-value was considered significant when it was <0.05.

Table 6: Abdominal aortic wall calcification in group X, Y and Z subjects (n=60)

Score	Age group (Years) (Number of the subject)			Statistical test result (t- value/p-value)	
	Group X	Group Y	Group Z	λ2-value/P-value	ANOVA-value/P-value
0 (n=06)	6	0	0	6.39/0.047	9.67/0.038
1 (n=22)	7	15	0	7.71/0.001	
2 (n=21)	2	16	3	3.33/0.027	
3 (n=11)	0	4	9	1.18/0.017	

P-value was considered significant when it was <0.05.

Table 7: Age of the participants according to the Density of Pineal calcification.

The density of pineal calcification	Mean±SD	Level of significance (F-test)	95%CI for mean		Group Comparison	Level of significance (t-test)	
			Lower	Upper			
0-50	59.2±8.41 691	0.017	53.1 789	65.2 211	-	0.436	
51-150	64.5±8.93 495		58.1 083	70.8 917	1.00 vs 2.00 (51-150)		
151-250	72.3333±2 .51661		66.0 817	78.5 849	1.00 vs 3.00 (151-250)		0.074
251-350	73.0±1.82 574		70.0 948	75.9 052	1.00 vs 4.00 (251-350)		0.029

Table 8: Association between Intradiscal calcification and density of pineal gland calcification.

Intradiscal calcification	The density of pineal calcification (0-250)				Statistical test result (P-value) (251-1000)
	N	%	N	%	
Yes	35	82.9	7	17.1	0.052
No	18	100	0	0	

P-value was considered significant when it was <0.05.

Table 9: Association between Posterior osteophyte and density of pineal gland calcification

Posterior osteophyte	The density of pineal calcification (0-250)				Statistical test result (P-value) (251-1000)
	N	%	N	%	
Yes	449	86.3	7	13.7	0.2
No	9	100	9	0	

P-value was considered significant when it was <0.05.

Table 10: Association between Disc height loss and density of pineal gland calcification

Disc height loss	The density of pineal calcification (0-250)				Statistical test result (P-value) (251-1000)
	N	%	N	%	
Yes	48	87.3	7	12.7	0.425
No	5	100	0	0	

P-value was considered significant when it was <0.05.

Table 11: Association between Abdominal aortic wall calcification and density of pineal gland calcification.

Aortic wall calcification	The density of pineal calcification (0-250)				Statistical test result (P-value) (251-1000)
	N	%	N	%	
Yes	47	87	7	13	0.359
No	6	100	0	0	

P-value was considered significant when it was <0.05.

DISCUSSION

This observational analytical study was carried out to evaluate the Correlation of lumbar intervertebral disc degeneration, abdominal aortic calcification on plain X-ray and Pineal gland calcification at CT in patients with low back pain. A total of 100 patients having low back pain were referred to the Department of

Radiology and Imaging of BIRDEM for X-ray of the lumbosacral spine from 1st January 2011 to 31st December 2012. 100 patients were enrolled first in this study. Ultimately 60 patients, who full filled the selection criteria were included. Their age ranges from 41 years to 74 years. After reviewing the clinical history demographic data, of all subjects were collected. A plain X-ray of the lumbosacral spine and CT scan of the

brain was performed for each patient. The findings of the study are discussed below based on the objective of the study considering the related previous studies. With the advance of age degenerative changes start in the human body. Degenerative changes are more marked in older than younger groups. Similar findings were revealed in the present study. The mean age was 61.26 years with a standard deviation of the mean (SD) #13.34 years and their age ranged from 41 to 74 years. The majority of the respondents were from age 51 to 70 years. A similar study was carried out by Turget et al. (1998) on 81 (66 women and 15 men) subjects: younger than 45 years (group X, n=22), 45-65 years of age (group Y, n=45), and older than 65 years (group Z, n = 14).^[16] In addition to clinical data, computed tomography (CT) scan of the brain as well as X-ray and CT examination of the lumbar spine. The mean age was 77.46 years with a standard deviation of the mean (SD) \pm 11.34 years and their age ranged from 40 to 80 years. Similarly, Benneker et al. (2005) showed mean age was 54 years with a range from 40 to 86 years.^[17] An almost similar age range was obtained by Adams et al (1984) and Yong, Alias & Shuaib (2003), where the authors showed an age range between 35 to 70 years and 45 to 71 years respectively, which closely resembled the current study.^[18,19] Under 40 years of age, the degree of disc degeneration varied among individuals and over the age of 60, most of the discs were markedly degenerated reported by Fujiwara et al. (1999).^[20] On the other hand, Pye et al. (2004) observed higher mean age in men and women having low back pain, which were 65.3 years and 65.2 years respectively.^[21] The higher age range may be due to increased life expectancy, geographical location and racial influences that

may have significant impacts on degrees of degenerative osteoarthritis. In this present study, it was observed that nearly two-thirds 61.7% of the subjects were male and 38.3% were female and the male-female ratio was 1.6:1. A similar identical male-female ratio was observed by Yong, Alias and Shuaib (2003).^[22] where the authors found the ratio was 1.7:1. Pye et al. (2004) mentioned that Osteophytes and intradiscal calcification were more frequent in men than women, but there was no gender difference in the frequency of disc space narrowing.^[21] Similarly, Schepper et al. (2010) have shown osteophytes were the most frequent radiographic feature observed, with men having the greatest frequency.^[23] Disc space narrowing was more frequent in women than men. Each group (X, Y, Z) were subdivided into four categories (Score 0, 1, 2, 3) based upon abdominal aortic wall calcification. The majority of the subjects (22 out of 60) had aortic wall calcification involving less than one-third area (Score 1) and subjects were mostly from the Y Group in this category. Less than half the area. (Score 2) involvement was observed in 21 subjects. More than two-thirds of the area (Score 3) involvement was seen in 11 subjects. A statistically significant difference in abdominal aortic wall calcification was revealed between Groups (Chi-square test) and among the groups (ANOVA test). A similar study was done by Mehmet et al. (1999).^[24] He showed The average value of the degree of aortic wall calcification in individuals from group Z (1.500 ± 0.292) was determined to be higher than in groups X (0.045 ± 0.045) and Y (0.533 ± 0.121), demonstrating a determination to be higher than in groups X (0.045 ± 0.045) and Y (0.533 ± 0.121). demonstrating an increase in atherosclerotic process with advanced age; the



difference among the groups was highly significant ($p < 0.001$, $p < 0.005$). In a cross-sectional observational study, Kurunlahti et al. (1999), found that there was a significant association between an atheromatous lesion in the abdominal aorta and low back pain.^[9] Most recently Leino-Arjas et al. (2006) reported that high serum lipids are responsible for radiating low back pain, consistent with the development of disc degeneration due to atherosclerosis.^[25] Disc height loss was measured in percentage and it was seen in the present study that the majority of the study subjects had 21-30% - disc height loss and they were scored as 2. In these 2 scoring groups, the majority of the subjects were from the Group of Y (51-70years) and female groups. More than 31% (Score 3) disc height loss was observed in 11 subjects of which six subjects were from Group Y and five from group Z. Less than 10%-disc height loss was seen among 5 subjects. Statistically, a significant difference was revealed between Groups (Chi-square test) and among the groups (ANOVA test). Pearson's correlation test showed a statistically significant relation between disc height loss with age groups. A related study was done by Turget et al. (1998).^[16] They showed a relation between the degree of disc degeneration, pineal gland calcification and abdominal aortic calcification. degenerative changes They also showed that the enhanced correlated with increased density of pineal gland calcification. The average values of disc degeneration scores obtained from X-ray studies in individuals from group Z were found to be higher (2.714 ± 0.429) compared to values in groups X and Y (0.373 ± 0.133 and 1.609 ± 0.225 respectively), indicating an increase in the degeneration process with advanced age. For X-ray scores, significant differences were detected by the dual comparisons of the groups (p

< 0.001 , $p < 0.001$ and $p < 0.05$). Importantly, arteriosclerotic circulation disorders of the intervertebral disc have discopathy and this hypothesis is also confirmed in the present study.^[10] Posterior osteophytes were observed as part of degenerative changes and it was seen that the majority (20 out of 60) of the subjects had pointed types of osteophytes (Score 1). In this group (Score 1) most of the subjects were within Group Y. In 17 subjects, the size of the osteophytes was less than 2. About 13 subjects had a score of 3 on basis of posterior osteophytes and subjects were from Group Z in this score mostly. Statistically, a significant difference was revealed between Groups (Chi-square test) and among the groups (ANOVA test). Similarly, Schepper et al. (2010) have shown osteophytes were the most frequent radiographic feature observed, having the greatest association with degenerative changes.^[26] In this study, 18 subjects had no intradiscal calcification. Rim calcification was observed in 22 subjects where most (17) subjects belong to the 51-70 (Group Y) age group. About 13 subjects were scored 2 on basis of intradiscal calcification. Statistically, a significant difference was revealed between Groups (Chi-square test) on basis of the presence of intradiscal calcification in different groups. Similarly, Pye et al. (2004) mentioned that intradiscal calcification was more frequent in association with degenerative changes which are induced by increased density of pineal gland calcification and atherosclerotic changes in the aorta.^[21] In the present study, a CT scan was performed on 30 subjects among 60. It was seen that majority of the subjects had the density of the Pineal gland ranging from +51 to +150 HU. Only 4 subjects had the density of the Pineal gland ranging from +351 to +1000 HU. A



statistically significant relation was observed between the age groups with the density of the Pineal gland in the ANOVA test. Meant SD density of the Pineal gland among of total 30 subjects was 136.98164.11 HU. In Group X, the density of Pineal Gland was 83.57+14.45 HU. The density of the Pineal gland was 134.65±13.23HU and 151.66121.32 HU in Group Y and Group Z respectively. Some parameters of the degenerative disc disease and aortic wall calcification were significantly related to the density of the Pineal gland. Similarly, Kunz et al (1999) obtained the size of the pineal calcification and the amount of the remaining active pineal tissue at a non-contrast CT scan of the brain.^[14] They showed the increased density of pineal gland calcification causes decreased Melatonin secretion. It was shown by Turget et al. (1998) that there was a significant interaction between pineal gland calcification and findings of lumbar intervertebral disc degeneration or abdominal aorta calcifying atherosclerosis.^[16] The average density of the calcified region was found to increase progressively with ageing, 81.33±11.38 in group X compared to 130.35±14.22 in group Y and 151.36120.14 in group Z. By statistical analysis, the difference between group Y and group Z was found to be significant ($p < 0.05$). It also has been reported by Humbert et al. (1994) that calcification of pinealocytes is a result of death due to the cellular regeneration process, resulting in decreased melatonin (MEL) production.^[27] A previous study reported that there is a correlation between the occurrence of pineal calcification and a reduced MEL production, but it is generally accepted that an increasing degree of pineal calcification with advanced age leads to decreasing MEL secretion.^[28]

Limitations of the study:

Although optimum care had been taken by the researcher in every step of this study, some limitations still exist. The study was conducted in a single center. So the study population may not be representative of the whole country. This study was limited by the small study sample. Because of the short duration of the study, a large sample could not be taken.

CONCLUSIONS

The study was undertaken to find out the Correlation between lumbar intervertebral disc degeneration, abdominal aortic calcification on plain X-ray and Pineal gland calcification at CT in low back pain subjects. The data obtained showed that the density of pineal gland calcification is statistically significant with increasing age. There was also a positive association between intradiscal calcification and density of pineal gland calcification, but no significant association among other parameters with the density of pineal gland calcification. As increased density of pineal gland calcification is a predictor of degenerative change and is associated with the degenerative process. It can be concluded in the present study that measurement of the density of pineal gland calcification at CT in low back pain subjects may be helpful for the early management of the degenerative process. However, further studies may be carried out by including a larger number of study subjects and other parameters of degenerative changes for evaluation of significant association among other parameters of the degenerative process with a density of pineal gland calcification.



REFERENCES

1. Buckwalter JA. Aging and degeneration of the human intervertebral disc. *Spine (Phila Pa 1976)*. 1995;20(11):1307-14. doi: 10.1097/00007632-199506000-00022.
2. Nerlich AG, Schleicher ED, Boos N. 1997 Volvo Award winner in basic science studies. Immunohistologic markers for age-related changes of human lumbar intervertebral discs. *Spine (Phila Pa 1976)*. 1997;22(24):2781-95. doi: 10.1097/00007632-199712150-00001.
3. Dhenain M, Ruffins SW, Jacobs RE. Three-dimensional digital mouse atlas using high-resolution MRI. *Dev Biol*. 2001;232(2):458-70. doi: 10.1006/dbio.2001.0189.
4. Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, et al. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science*. 2001;294(5550):2348-51. doi: 10.1126/science.1067179.
5. Lamarre D, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, et al. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature*. 2003;426(6963):186-9. doi: 10.1038/nature02099.
6. Andrade C, Santos JA, Pinto JG, Corte-Real J. Large-scale atmospheric dynamics of the wet winter 2009–2010 and its impact on hydrology in Portugal. *Clim Res*. 2011;46(1):29-41.
7. Benneker LM, Heini PF, Anderson SE, Alini M, Ito K. Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration. *Eur Spine J*. 2005;14(1):27-35. doi: 10.1007/s00586-004-0759-4.
8. Weiler C, Schietzsch M, Kirchner T, Nerlich AG, Boos N, Wuertz K. Age-related changes in human cervical, thoracic and lumbar intervertebral disc exhibit a strong intra-individual correlation. *Eur Spine J*. 2012;21 Suppl 6(Suppl 6):S810-S818. doi:10.1007/s00586-011-1922-3
9. Kurunlahti M, Tervonen O, Vanharanta H, Ilkko E, Suramo I. Association of atherosclerosis with low back pain and the degree of disc degeneration. *Spine (Phila Pa 1976)*. 1999;24(20):2080-4. doi: 10.1097/00007632-199910150-00003.
10. Kauppila LI, Penttilä A, Karhunen PJ, Lulu K, Hannikainen P. Lumbar disc degeneration and atherosclerosis of the abdominal aorta. *Spine (Phila Pa 1976)*. 1994;19(8):923-9. doi: 10.1097/00007632-199404150-00010.
11. Reiter B, Pfeifer U, Schwab H, Sessitsch A. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl Environ Microbiol*. 2002;68(5):2261-2268. doi:10.1128/AEM.68.5.2261-2268.2002
12. Adeloje A, Felson B. Incidence of normal pineal gland calcification in skull roentgenograms of black and white Americans. *Am J Roentgenol Radium Ther Nucl Med*. 1974;122(3):503-7. doi: 10.2214/ajr.122.3.503.
13. Gilbert JD, Manica A. Parental care trade-offs and life-history relationships in insects. *Am Nat*. 2010;176(2):212-26. doi: 10.1086/653661.
14. Angulo C, Arnould M, Rayet M, Descouvemont P, Baye D, Leclercq-Willain C, et al. A compilation of charged-particle induced thermonuclear reaction rates. *Nuclear Physics A*. 1999;656(1):3-183.
15. Wedel B, Humbert P, Harteneck C, et al. Mutation of His-105 in the beta 1 subunit yields a nitric oxide-insensitive form of soluble guanylyl cyclase. *Proc Natl Acad Sci U S A*. 1994;91(7):2592-2596. doi:10.1073/pnas.91.7.2592
16. Escolà A, Planas S, Rosell JR, Pomar J, Camp F, Solanelles F, et al. Performance of an ultrasonic ranging sensor in apple tree canopies. *Sensors (Basel)*. 2011;11(3):2459-77. doi: 10.3390/s110302459.
17. Benneker LM, Heini PF, Anderson SE, Alini M, Ito K. Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration. *Eur Spine J*. 2005;14(1):27-35. doi: 10.1007/s00586-004-0759-4.
18. Adams NG, Smith D, Millar TJ. The importance of kinetically excited ions in the synthesis of interstellar molecules. *J R Astron Soc Can*. 1984;211(4):857-65.
19. Kohat AK, Kalita J, Ramanivas S, Misra UK, Phadke RV. Clinical significance of magnetic resonance imaging findings in chronic low backache. *Indian J Med Res*. 2017;145(6):796-803. doi:10.4103/ijmr.IJMR_1653_14



20. Sekine K, Ohuchi H, Fujiwara M, Yamasaki M, Yoshizawa T, Sato T, et al. Fgf10 is essential for limb and lung formation. *Nat Genet.* 1999;21(1):138-41. doi: 10.1038/5096.
21. Wallace VP, Fitzgerald AJ, Shankar S, Flanagan N, Pye R, Cluff J, et al. Terahertz pulsed imaging of basal cell carcinoma ex vivo and in vivo. *Br J Dermatol.* 2004;151(2):424-32. doi: 10.1111/j.1365-2133.2004.06129.x.
22. Yong PY, Alias NN, Shuaib IL. Correlation of clinical presentation, radiography, and magnetic resonance imaging for low back pain-A preliminary survey. *HKJR.* 2003; 6:144-51.
23. Olmer R, Haase A, Merkert S, Cui W, Palecek J, Ran C, et al. Long term expansion of undifferentiated human iPS and ES cells in suspension culture using a defined medium. *Stem Cell Res.* 2010;5(1):51-64. doi: 10.1016/j.scr.2010.03.005.
24. Taylor DL, Edwards AD, Mehmet H. Oxidative metabolism, apoptosis and perinatal brain injury. *Brain Pathol.* 1999;9(1):93-117. doi: 10.1111/j.1750-3639.1999.tb00213.x.
25. Kivimäki M, Leino-Arjas P, Kaila-Kangas L, Luukkonen R, Vahtera J, Elovainio M, et al. Is incomplete recovery from work a risk marker of cardiovascular death? Prospective evidence from industrial employees. *Psychosom Med.* 2006;68(3):402-7. doi: 10.1097/01.psy.0000221285.50314.d3.
26. Scheele J, de Schepper EI, van Meurs JB, Hofman A, Koes BW, Luijsterburg PA, et al. Association between spinal morning stiffness and lumbar disc degeneration: the Rotterdam Study. *Osteoarthritis Cartilage.* 2012;20(9):982-7. doi: 10.1016/j.joca.2012.05.011.
27. Wedel B, Humbert P, Harteneck C, Foerster J, Malkewitz J, Böhme E, et al. Mutation of His-105 in the beta 1 subunit yields a nitric oxide-insensitive form of soluble guanylyl cyclase. *Proc Natl Acad Sci U S A.* 1994;91(7):2592-6. doi: 10.1073/pnas.91.7.2592.
28. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res.* 2005;52(4):313-20. doi: 10.1016/j.phrs.2005.05.004.

Source of Support: Nil, Conflict of Interest: None declared