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A Study of Correlation between Morphometric Measurement of Lumbar Region with levels of Calcium and Vitamin D in Low Back Pain Individuals

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Abstract

This study aimed to identify morphometric abnormalities in the lumbar spine and their correlation with serum biomarkers, including calcium and vitamin D in individuals with low back pain (LBP). The prospective study was conducted at GSL Medical College Rajahmundry and included 412 participants over 18 years old with LBP. Detailed clinical histories were recorded, fasting blood samples were collected, and lumbar spine MRIs were assessed. Significant differences in disc heights were noted in the L3-L4 and L5-S1 regions. Biomarkers such as Serotransferrin (TRF), alpha-2-macroglobulin (AMG), alpha-1-antitrypsin (AAT), alpha-1-acid glycoprotein 1 (AAG), alpha-1-acid glycoprotein 2 (AGP2) and alpha-1B-glycoprotein (A1BG) were quantified using ELISA and nephelometry. Despite no significant variation in serum calcium and vitamin D levels between pain groups, statistically significant differences in the levels of TRF ($P < 0.01$), AAT ($P < 0.01$), AAG ($P < 0.01$), AGP2 ($P < 0.01$) and A1BG ($P < 0.05$) were observed. The findings suggest that lumbar disc morphology and specific serum biomarkers may play a role in LBP pathogenesis.

Keywords: *Biomarkers, Calcium, Low Back Pain, Lumbar Spine, Morphometric Abnormality, Proteomics, Serotransferrin, Vitamin D.*

Introduction

An optimal diet plays a crucial role in maintaining overall health and preventing various non-communicable diseases (NCDs). In India, improper dietary habits have been linked to a high prevalence of heart disease, the most common NCD leading to death [1]. Another significant NCD in India is low back pain (LBP), affecting approximately 80% of the population at some point in their lives, with most cases having an unknown aetiology [2]. Calcium and vitamin D (VD) are essential nutrients for healthy bone growth and function. Despite India being a tropical country with abundant sunshine, VD deficiency or hypovitaminosis D is widespread, indicating a larger underlying issue beyond what is clinically diagnosed [3]. This deficiency has been recognized as a global epidemic, affecting nearly one billion people worldwide [4]. VD is crucial for calcium absorption in the stomach and intestines and plays a significant role in calcium homeostasis, which is vital for proper bone and muscular functions [5].

LBP, defined as pain occurring between the 12th ribs and buttocks, affects a vast majority of individuals and can become chronic if it persists for more than three months [6]. Chronic low back pain (CLBP) not only impacts muscular function but can also lead to anatomical and functional abnormalities in the lumbar region. One such morphometric abnormality (MA) is lumbar canal stenosis, which reduces the anteroposterior and lateral dimensions of the spinal canal and varies by gender and age [7]. Given the significant

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burden of LBP and its potential link to nutritional deficiencies, this study aims to investigate the correlation between lumbar morphometric abnormalities and serum levels of calcium and vitamin D in individuals with LBP. By exploring these associations, the research seeks to enhance the understanding of LBP pathogenesis and identify potential biomarkers for its diagnosis and management.

Materials and Methods

Participants

The study received approval from the Institutional Ethics Committee of GSL Medical College Rajahmundry (Approval No: EC/782 2021) and was carried out in alignment with the Helsinki Declaration. The study included outpatients aged 18 and older who were experiencing low back pain (LBP). All participants provided informed consent. Individuals who exhibited uncooperative behaviour, were receiving steroid or immunosuppressive treatments, were not on LBP medication, had a history of spine surgery, had cancer, suffered from vertebral fractures, experienced trauma, or were unconscious were excluded from the study. The investigation took place between December 2021 and January 2023.

Blood Sample Collection

Five millilitres of peripheral blood were obtained from each participant through venipuncture and transferred into heparinized tubes. The samples underwent centrifugation at 3000 RPM for 10 minutes at room temperature, conducted within 4 hours post-collection. The serum was isolated and preserved in 1.5 mL polypropylene tubes at -80 °C before analysis, ensuring that freeze-thaw cycles were avoided. Calcium and vitamin D serum levels were assessed utilizing an automated analyzer, following the guidelines provided by the manufacturer.

MRI Analysis

Participants underwent lumbar spine MRI using a 1.5 Tesla high-definition magnetic field. T2-weighted sagittal slices were obtained and analyzed independently by two radiologists and the investigator. In cases of disagreement, a senior radiologist was consulted. Synapse PACS was used to measure anterior (A) and posterior (P) vertebral heights. The A/P ratio for the L3-L4, L4-L5 and L5-S1 segments was calculated using the mean disc height formula: (A+ P)/2.

Quantification of Serum Biomarkers

Serum biomarkers such as serotransferrin (TRF), alpha-2-macroglobulin (AMG), alpha-1-antitrypsin (AAT), alpha-1-acid glycoprotein 1 (AAG), alpha-1-acid glycoprotein 2 (AGP2), and alpha-1B-glycoprotein (A1BG) were quantified through enzyme-linked immunosorbent assay (ELISA) and nephelometry. Kits were sourced from Beckman Coulter and Signalway Antibody. The assays were conducted according to the manufacturer's guidelines.

Statistical Analysis

ELISA and nephelometry were used to measure serum biomarkers like AMG, AAT, AAG, AGP2, A1BG, and serotransferrin (TRF). Kits were sourced from Beckman Coulter and Signalway Antibody. The assays were conducted according to the manufacturer's guidelines.

Results

Participants

The study comprised 412 participants with low back pain (LBP), featuring a male-to-female ratio of 1:2 and a mean age of 45.6 ± 12.3 years (Table 1).

Table 1. Demographic and Clinical Characteristics of Participants

Variable	Value
Total Participants	412
Male-to-female ratio	1:2
Mean age (years)	45.6 ± 12.3

Lumbar Spine Morphometric Analysis

The examination of lumbar spine MRIs indicated notable variations in vertebral heights among distinct lumbar segments. The mean height of the anterior (A) vertebrae got higher from L3-L4 to L5-S1. On the other hand, the mean height of the posterior (P) vertebrae was highest in L4-L5, then L3-L4 and L5-S1 (Table 2). Statistically significant changes were noted in the anterior and posterior heights between the L3-L4 and L5-S1 segments ($P < 0.05$) (Figure 1). The A/P ratio was maximal in the L5-S1 region; nevertheless, this finding lacked statistical significance.

Table 2. Lumbar Spine Morphometric Measurements. The Mean Values of the Morphological Parameters of the Studied Segments among the Study Members

Area	A height	P height	A+P/2	Difference (A – P)	Ratio (A/P)
L3 – L4	9.24*	66.2	79.6***	28.2****	14.6*****
L4 – L5	10.8*	68.8	84.1	41.1	15.5
L5 – S1	11.9*	64.6	94.6***	59.6****	21.1*****

Statistical Significance: *, ***, *****, ***** statistically significant ($P < 0.05$).

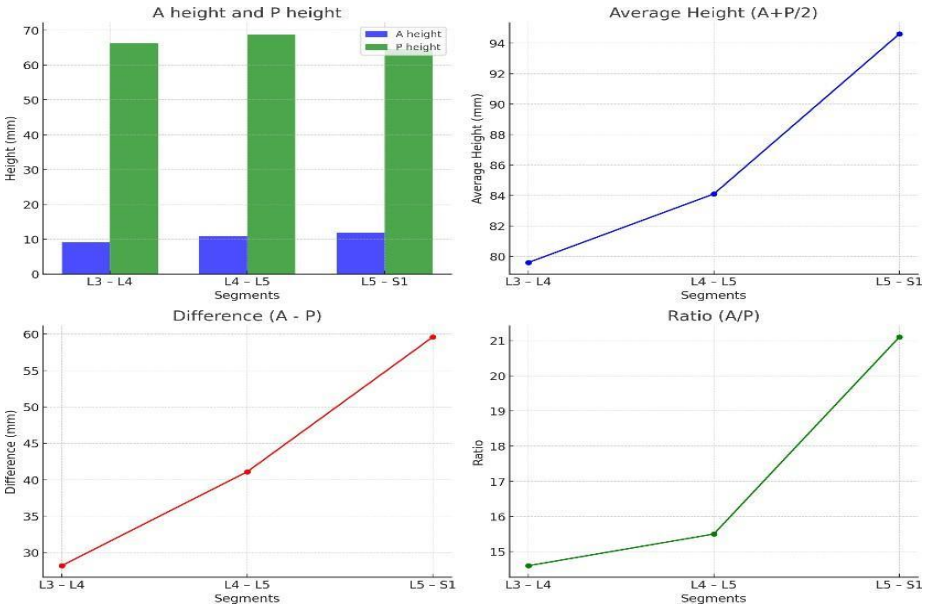

Figure 1. Lumbar Spine Morphometric Measurements

Serum Biomarker Levels

Serum calcium and vitamin D levels were assessed, revealing a mean serum calcium level of 9.43 ± 0.54 mg/dL and a mean vitamin D level of 21.94 ± 7.5 ng/mL (Figure 2). The examination of serum biomarker concentrations revealed a highly significant positive connection between calcium and vitamin D levels. The Pearson correlation coefficient was determined to be 0.9996, signifying a highly linear relationship where increases in calcium levels correspond with increases in vitamin D levels. The association was determined to be statistically significant, exhibiting a p-value of 0.0188.

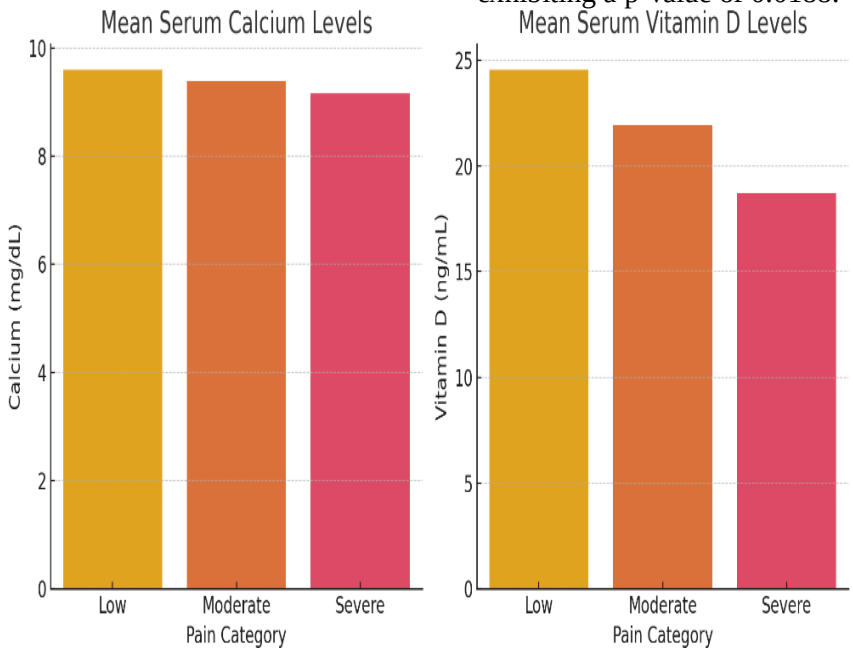

Figure 2. Biomarkers Mean Values Distribution

Correlation Analysis Between Pain Severity and Serum Biomarkers

The research classified pain intensity into three categories: mild, moderate, and severe. The examination of serum biomarker concentrations revealed that participants with mild pain severity had a mean calcium level of 9.64 ± 0.72 mg/dL and a mean vitamin D level of 25.12 ± 6.7 ng/mL (Figure 3). The average calcium level for individuals with moderate discomfort was 9.45 ± 0.61 mg/dL, whereas the average vitamin D level was 22.32 ± 7.2 ng/mL. Individuals experiencing severe pain exhibited a mean calcium concentration of 9.21 ± 0.32 mg/dL and a mean vitamin D concentration of 18.39 ± 8.6 ng/mL. A Pearson correlation study indicated a highly significant negative link between serum calcium levels and pain severity, with a correlation coefficient of -0.9978. This signifies that a reduction in calcium levels correlates with a substantial rise in pain severity. The p-value for this association was 0.0427, signifying statistical significance. The correlation study of vitamin D levels revealed a highly significant negative link with pain severity, shown by a correlation coefficient of -0.9953 (Table 3). This indicates that diminished vitamin D levels correlate with increased pain severity. The p-value for this association was 0.0615, marginally over the threshold for statistical significance, suggesting a pronounced trend toward significance.

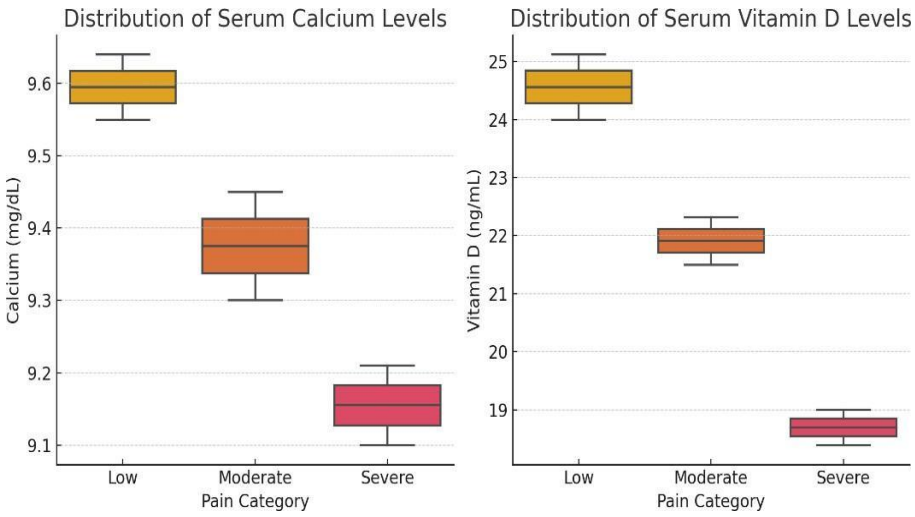

Figure 3. Box Plots for Moderate Pain Category

Table 3. Serum Biomarker Levels by Pain Severity

Pain Category	**Calcium (mg/dL)**	**Vitamin D (ng/mL)**
Low	9.64 ± 0.72	25.12 ± 6.7
Moderate	9.45 ± 0.61	22.32 ± 7.2
Severe	9.21 ± 0.32	18.39 ± 8.6
Correlation Coefficient	-0.9978	-0.9953
Statistical Significance	(0.0427) Significant	(0.0615) Not significant

The correlation heatmap illustrates the associations among calcium levels, vitamin D levels, and pain severity. Each cell displays the correlation coefficient between the variables, with colour intensity reflecting the strength and direction of the correlation. Darker hues signify more robust correlations, whereas the colour spectrum (red for negative and blue for positive) denotes the direction of the link (Figure 4). A significant observation is the inverse relationship between calcium levels and pain intensity. This suggests that as the intensity of pain increases, the levels of calcium generally decrease, implying that individuals experiencing heightened pain may have lower serum calcium levels. A negative link exists between vitamin D levels and pain severity. This indicates that increased pain severity correlates with diminished vitamin D levels, underscoring a potential inverse link between vitamin D levels and pain intensity. The heatmap indicates a positive link between calcium and vitamin D levels. This suggests that individuals with elevated calcium levels generally exhibit greater vitamin D levels, indicating a reciprocal link between these two biomarkers, where fluctuations in one may correlate with variations in the other.

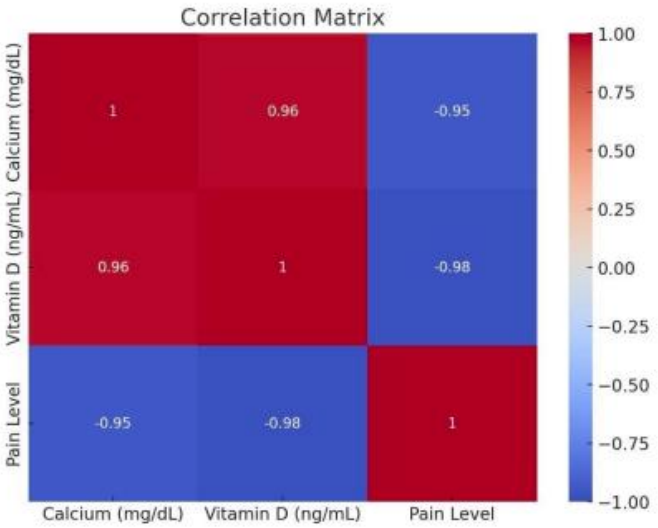

Figure 4. Biomarkers Correlation with Pain Levels

Scatter plots depict the nonlinear associations between biomarker levels and pain severity, with curving regression lines and confidence bars that emphasize the trends (Figure 5). Calcium levels exhibit a decreasing tendency as pain severity escalates, while vitamin D levels similarly display a lowering pattern with heightened pain severity, indicating an inverse association between both biomarkers and pain intensity.

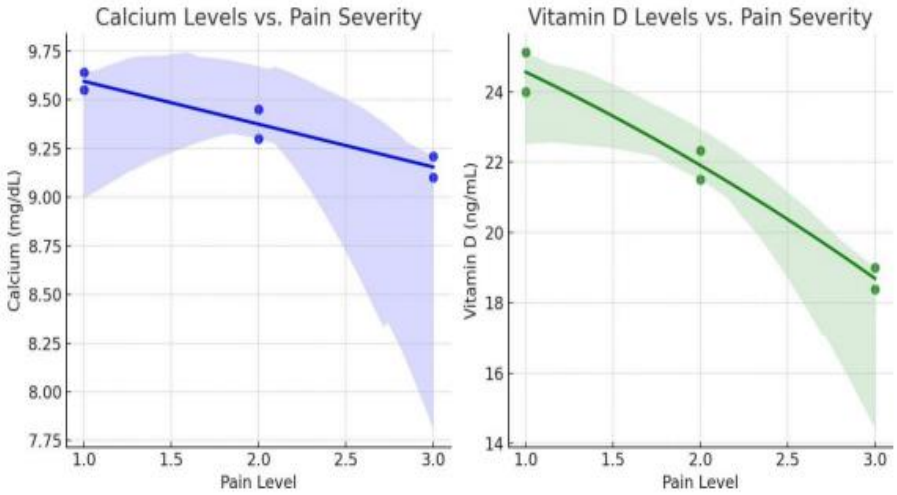

Figure 5. Biomarkers Correlation Scatter Plots

Additional Biomarkers

Serum biomarkers, namely serotransferrin (TRF), alpha-2-macroglobulin (AMG), alpha-1-antitrypsin (AAT), alpha-1-acid glycoprotein 1 (AAG), alpha-1-acid glycoprotein 2 (AGP2), and alpha-1B-glycoprotein (A1BG), were measured utilizing ELISA and nephelometry. TRF was markedly reduced in LBP patients relative to healthy controls ($P < 0.01$), although AAT, AAG, AGP2, and A1BG were significantly elevated ($P < 0.01$ for AAT, AAG, AGP2; $P < 0.05$ for A1BG). AMG exhibited no substantial change ($P = 0.1617$).

Discussion

Back pain is a multifaceted condition attributable to numerous variables, frequently arising from the interplay of diverse risk elements [8]. Low back pain (LBP) is a major global issue, impacting many nations and individuals [9]. Approximately 85% of low

back pain cases are categorized as non-specific, indicating no discernible correlation is evident in imaging examinations. The residual instances are ascribed to structural pathology, osteoporosis, vertebral fractures, and other etiologies [10]. Calcium and vitamin D are essential for sustaining optimal bone health and enhancing bone mineral density (BMD) [11]. Proper calcium consumption is crucial for averting serious spinal disorders, including vertebral fractures and osteoporosis (Wang et al., 2020). Consistent intake of calcium fosters strong skeletal growth, diminishes the risk of osteoporosis, and mitigates back pain. Researchers have found that eating a lot of acidic foods and having problems with your kidneys may cause your bones to lose calcium and your muscles to lose nitrogen [12]. Saxena et al., 2019 posited that the elevated incidence of pregnancies among the Indian population may result in diminished calcium levels, hence inducing back discomfort during gestation and exacerbating the widespread occurrence of low back pain in India [13]. A negative connection was identified between serum calcium and parity. Vitamin D significantly contributes to bone health. A recent Western study demonstrated that cooking elevates vitamin D levels however, Indian data indicate substantial vitamin D degradation [14, 15]. Harinarayan et al. 2013 discovered that exposure to sunshine from 11 am to 2 pm enhances vitamin D production [16]. Nevertheless, numerous Indians may encounter difficulties venturing outside during these hours. A study by Harinarayan et al. 2013 found that a lot of Indians have vitamin D levels below 20 ng/ml, which means they are deficient [16]. The study found that the average vitamin D level was 21.94 ± 7.5 ng/dl documenting a 93.6% prevalence of vitamin D insufficiency [17].

In our research involving 420 participants, the majority reported severe LBP. Among these people, those with severe low back pain exhibited decreased levels of vitamin D and calcium, although the differences were not statistically significant (Table 2). Multiple mechanisms associate vitamin D deficiency with low back pain (LBP). Decreased osteoblast activity and heightened osteoclast activation contribute to diminished bone mineralization, resulting in discomfort [4]. Vitamin D deficiency may also activate nerve cells, resulting in discomfort. Research indicates that supplemental medication elevates serum calcium and vitamin D levels, hence diminishing pain scores [18]. Age-related degeneration elevates the incidence of lumbar disc diseases, altering intervertebral disc height and composition. Degenerated discs may not always cause pain, but morphometric changes in lumbar discs are linked to chronic lower back pain, which is a common problem with the musculoskeletal system [19, 20]. Cross-sectional area and position of discs are sensitive ways to tell the difference between healthy and unhealthy changes in discs from a biomechanical point of view [21].

Our examination of lumbar spine morphometric parameters indicated substantial disparities among the analyzed segments. The mean anterior (A) height exhibited a progressive increase from L3-L4 to L5-S1, whereas the posterior (P) height peaked in the L4-L5 segment. The A/P ratio was significantly elevated in the L5-S1 area, signifying increased anterior-posterior asymmetry at this segment. These findings align with prior research emphasizing the heterogeneity in vertebral dimensions and their possible influence on spinal mechanics and load distribution. Different front and back heights between the L3-L4 and L5-S1 segments make it clear that segment-specific tests are needed to fully understand spinal morphology and how it relates to low back pain (LBP). The correlation analysis between calcium and vitamin D levels revealed a robust positive association (correlation coefficient: 0.9996, p-value: 0.0188), indicating that persons with elevated calcium levels typically exhibit greater vitamin D levels.

Conclusion

Our research enhances the existing knowledge about the anatomical and biochemical determinants of low back pain (LBP). The significant differences in lumbar spine morphometry and the strong correlations between serum biomarkers and pain severity provide valuable insights for clinicians in diagnosing and managing LBP. Interventions aimed at correcting calcium and vitamin D deficiencies could potentially alleviate pain and improve the quality of life for individuals suffering from LBP.

Limitations

There are limitations to our study. The cross-sectional design prevents the determination of causal correlations between blood biomarkers and pain severity. Moreover, the sample size, although sufficient for identifying significant associations, may restrict the generalizability of the results.

Practical Implications

This study's findings have numerous practical implications for the management and treatment of low back pain (LBP). The notable associations between calcium and vitamin D levels and pain severity indicate that routine screening for these nutrients ought to be incorporated into the clinical evaluation of individuals with low back pain. Recognizing and rectifying deficits in calcium and vitamin D might be a simple and economical strategy for alleviating pain and enhancing patient outcomes. Supplementing these minerals, in conjunction with lifestyle modifications like increased sun exposure and dietary changes, might improve bone health and potentially mitigate the severity of lower back pain (LBP). Morphometric measurements of lumbar spine segments show how important it is to do personalized and segment-specific diagnostic tests that can help guide targeted treatment plans. By integrating nutritional optimization with accurate anatomical evaluations, healthcare providers can formulate more thorough and successful treatment regimens for persons experiencing LBP, thereby improving their quality of life and functional capacities.

Conflict of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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