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Research Article

Association of selected inflammatory markers and risk factors with pain in patients undergoing cervical or lumbar disc herniation

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Abstract

Introduction: Radicular pain following disc herniation may be due to local or systemic inflammatory responses than nerve compression. This study assessed the association of serum interleukin-6 (IL-6) levels, high-sensitivity C-reactive protein (hs-CRP) and related risk factors (occupational and behavioural) in patients undergoing discectomy following cervical disc herniation (CDH) or lumbar disc herniation (LDH). Methods: The study recruited 77 patients from the main tertiary care hospital in Sri Lanka with cervical or lumbar pain and undergoing discectomy for LDH or CDH. Patients with any type of tissue/muscle injury not related to disc herniation, and those having acute and chronic infections were excluded. Sociodemographic information was gathered using a questionnaire. IL-6 and hs-CRP levels were measured using ELISA and an auto-analyzer respectively. Independent sample t-test and Pearson correlation coefficient were used for statistical analysis considering 0.05 significance level. Results: There was no significant correlation between serum IL-6 and hs-CRP levels (p=0.86) among patients with acute and chronic CDH and LDH. Patients with chronic neck/back pain showed significantly higher IL-6 levels (p=0.043) than those with acute neck/back pain. Serum hs-CRP levels were significantly higher (p=0.048) in patients with acute neck/back pain than patients with chronic pain. There was no significant association between; the nature (p=0.542) and duration (p=0.446) of occupation, type of exercise (p=0.371), and type of sports (p=0.339) in either CDH or LDH patients. Conclusion: The absence of a substantial link between IL-6, hs-CRP, CDH, or LDH indicates that these inflammatory biomarkers may not directly influence the development of CDH or LDH. However, increased IL-6 levels in patients with chronic pain indicates a significant association between IL-6 and chronic pain. Elevated hs-CRP levels in acute pain patients suggest a distinct inflammatory response specific to acute pain. Occupation, type of exercise and type of sports demonstrated no significant association with CDH or LDH.

Keywords: Cervical disc herniation, Lumbar disc herniation, Interleukin, High sensitivity C reactive protein

Introduction

Intervertebral disc herniation is a common disorder that affects nearly 5% of the global population. Though it is a multifactorial disease the pathophysiology is poorly understood.

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However, studies have reported environmental factors, constitutional factors, behavioural and genetic factors as the main determinants of disc herniation [1,2]. Disc herniation is most commonly seen in the lumbar region, followed by the cervical region [3,4]. Pain in the lower back, thigh, foot and toe are the main symptoms caused by lumbar disc herniation (LDH). When LDH compresses the sciatic nerve it will lead to the common medical problem sciatica, whereas cervical disc herniation (CDH) causes neck pain and upper limb radiculopathy [4].

The intervertebral disc tissue is a cartilaginous structure composed of an inner gelatinous nucleus pulposus (NP) and an outer fibrous ring, the annulus fibrosus. Herniation of NP beyond the limits of intervertebral disc space gives rise to mechanical compression of the spinal nerve causing nerve radiculopathy which results in pain [5,6]. The direct contact between NP and nerve root induces inflammation reactions resulting in inflammatory cell activation, infiltration, attraction of leukocytes and vascular permeability changes in surrounding tissues leading to up-regulation of inflammatory markers such as interleukins (ILs), nitric oxide, prostaglandins, tumor necrosis factor, and matrix metalloproteinase around the herniated disc tissue. Studies have found elevated Interleukins (IL) mainly IL-6, IL-8 and IL-2 in patients with radiculopathy [7]. Elevated inflammatory markers cause a systemic inflammatory response which in turn elevates the synthesis of acute phase proteins such as C-reactive proteins (CRP) and high sensitivity CRP (hs-CRP). The elevation of CRP as a response to early stages of inflammation is regarded as a valuable laboratory marker in assessing inflammation. IL-6 causes activation and increased secretion of hs-CRP, where IL-6 acts as an inducer and gene expression regulator in the production of hs-CRP. Therefore, the influence of pain and inflammation around the herniated disc may be characteristic of the increase in acute pain related bio markers such as hs-CRP and IL-6 in blood [8]. However, subtle changes in local inflammation caused around the herniated disc area may not be detected by estimating CRP, hence hs-CRP has been recommended. Studies have found an elevation of hs-CRP in patients with disc herniation, especially in patients presenting with acute pain [9,10]. It is believed that the clinical presentation of radiculopathy is due to the mechanical compression of nerve roots by the herniated disc tissue. However, there is evidence where patients without mechanical compression have presented with severe radiculopathy and some do not complain about radiculopathy even with a large herniation. Therefore, radiculopathy caused by herniation does not completely explain the pathophysiology of pain associated with disc herniation [2]. Patients who are affected with disc herniation complain of acute as well as chronic pain. Acute pain usually subsides with analgesics and surgical interventions. However, chronic pain remains even after surgical intervention. Importantly, patients with disc herniation repeatedly complain of pain even after surgical intervention. Further, we hypothesized that pain and associated radiculopathy could be attributed to inflammatory biochemical markers secreted by the herniated disc tissue rather than the compression of the nerve. Further, there is compelling evidence indicating that degenerated discs can induce the expression of macrophages releasing especially IL-6 that triggers the secretion of CRP. Hence, the present study was conducted to investigate the association of high sensitivity C reactive proteins (hs-CRP) and IL-6 in patients with acute and chronic neck pain or back pain. Further, we also investigated the association of some selected behavioural and occupational factors related to CDH and LDH. More importantly little is known about the association of inflammatory biomarkers with disc herniation in Sri Lankan clinical settings which remains a major strength of the study.

Methods

Study design and study setting

The study was a descriptive cross-sectional study where patients who underwent surgical discectomy for CDH or LDH in a neurosurgical unit, at the main tertiary care hospital of Sri Lanka were recruited to the study.

Study population

A total of 77 consecutive patients with CDH or LDH who underwent surgical discectomy were randomly recruited after detailing the study protocol. The study included patients between 18-70 years of age, and both males and females. The patient's level of pain was rated using a visual analog scale. Informed written consent was obtained from all participants. Disc herniation was confirmed with Magnetic Resonance Image (MRI) by a consultant neurosurgeon and consultant radiologist. Patients with neck pain or back pain for less than three months duration were categorized as patients with acute neck or back pain while patients with pain for more than three months durations were regarded as patients with chronic neck or back pain. Patients with any type of tissue/muscle injury not related to disc herniation, with acute or chronic infections were excluded from the study. All patients who fulfilled inclusion criteria and consented for participation were included in the study.

Sample size calculation

The sample size was determined according to Charan J and Biswas T (2013) considering IL-6 reference range values according to the given formula [11].

$$N = \frac{Z^2 SD^2}{d^2}$$

N= Sample size
Z=1.96

IL-6 reference range 5-15pg/dl [12]
SD= $\frac{range}{4}$ = 2.5
d=0.56
N= 77

Sample collection

Approximately 5 mL of venous blood sample was collected prior to the surgery while adhering to all standard precautions, under aseptic, sterile conditions. Serum was separated after centrifugation at 3000 rpm for five minutes and was stored at -20°C for the investigation of inflammatory markers.

Assay of high sensitivity C-reactive protein

Serum hs-CRP was measured using the automated colourimetric method using Konelab 20 XT clinical analyser (Thermo Fisher Scientific, Finland) and the manufacturer's reagents and kit protocols were used for the analysis. CRP standard super high calibrator and CRP control high (Biolabo, France) were used to prepare the calibration curve while CRP HS calibrator (Thermo Scientific Co, Finland) was used for the preparation of hs-CRP calibration curve.

Serum IL-6 estimation

Serum IL-6 levels were analyzed using utilizing the sandwich enzyme-linked immunosorbent assay method (Elabscience Diagnostics) [13].

Data analysis

Data were entered and analyzed using standard statistical software SPSS version 25.0, IBM, USA. Socio-demographic data, risk factors (occupational and behavioural) and biochemical data were subjected to descriptive data analysis. Significance was determined using the p-value, with $p<0.05$ indicating a significant difference or association. Independent sample t-test was used to measure the significance of the difference between biochemical markers. Pearson correlation coefficient was employed to assess the correlation between two continuous variables. The correlation was measured at the significance level of 0.05.

Ethical approval

Ethical approval was obtained from the Ethics Review Committee of the Faculty of Medical

Sciences, University of Sri Jayewardenepura, Colombo, Sri Lanka (Ref No: MLS/07/18).

Results

Characteristics of study subjects

The study sample consisted of 77 patients where 27 (35.1%) presented with CDH, and 64.9% had LDH. There were 54.5% (n=42) males, and 45.5% (n=35) females. Mean age of the participants was 49.5±12.9 years. The mean body mass index (BMI) was 23.8±3.8 kg/m^2. More than half the number of patients (53.2%, n=41) were overweight (>23 kg/m^2) while there were 6.5 % (n=5) underweight patients (<18.5 kg/m^2). The majority of the patients (62.3%, n=48) suffered from chronic neck or back pain while the rest of the patients had acute neck or back pain.

Analysis of behavioral and occupational risk factors among CDH and LDH subjects

The majority of the patients with CDH were involved in light physically demanding occupations (51.8%) while patients with LDH were involved in heavy physically demanding occupations (50%). However, there was no significant association between CDH and LDH subjects regarding the physically demanding nature of occupation (p=0.542), duration of occupation (p=0.446), type of exercise (p=0.371), and type of sports involved (p=0.339). Further, there was no significant difference between the habit of smoking (p=0.629) of CDH and LDH subjects (Table 1).

Analysis of IL-6 and hs-CRP levels among patients with chronic or acute pain

Patients with chronic neck/back pain (n=48) showed significantly (p=0.043) higher IL-6 levels (197.61 pg/mL) compared to patients with acute neck/back pain (119.65 pg/mL, n=29). Significantly higher (p=0.048) hs-CRP levels were observed in patients with acute neck or back pain (8.82 mg/L) compared to that of patients with chronic neck or back pain (3.50 mg/L) (Table 2). Further, a significant correlation was not found between serum IL-6 and hs-CRP levels in patients with disc herniation (r=0.02, p=0.86) (Figure 1).

Discussion

Disc herniation is an injury that occurs in intervertebral disc materials. It results in several symptoms such as low back pain, neck pain, sensory changes (numbness, tingling) and inflammation around the herniated area. Studies have shown that displacement or herniation of the disc material is influenced by several behavioral, environmental, biochemical and genetic factors [5,6]. In the present study, an association of inflammatory biomarkers such as serum IL-6 and hs-CRP levels in CDH and LDH patients who underwent surgical discectomy was evaluated. In addition, the association of CDH and LDH with selected socio-demographic factors such as age, sex, duration of pain, BMI, type of exercise, type of sports, physically demanding nature of occupation, and duration of occupation were also analyzed. Among the subjects, the majority were males 42 (54.5%) and the mean age of the participants was 49.5±12.9 years. A systematic review found that the highest prevalence of lumbar disc herniation was among people between 30-55 years of age which is consistent with the present study results [5]. Vialle et al (2010) have suggested that the mean age for disc herniation is 37 years, while another study had reported the mean age as 45±13 years and majority of cases were males [14,15]. Further supportive studies indicated similar mean ages for disc herniation as 42±10 years [16] and 41±10 years [17]. Thus, the mean age of the present study is compatible with the reported findings. It is suggested that disc herniation is not pronounced in the early two decades of life but peaks in the fourth decade. It is evident that with age, the content of the disc, proteoglycan and water level gradually decreases and collagen content increases. This composition changes in the disc leads to a change in its

Table 1: Selected occupational and behavioural risk factors and their association with cervical disc herniated and lumbar disc herniated subjects

Variables	CDH patients (%) n=27	LDH patients (%) n=50	p value
Level of physically demanding nature of the occupation			0.542
Light	3 (11.1)	5 (10)	
Moderate	14 (51.9)	20 (40)	
Heavy	10 (37.0)	25 (50)	
Duration of occupation (in years)			0.446
1-10	4 (14.8)	8 (16)	
11-20	7 (25.9)	19 (38)	
21-30	9 (33.3)	12 (24)	
31-40	4 (14.8)	10 (20)	
41-50	2 (7.4)	1 (2)	
> 50	1 (3.7)	0	
Type of exercise			0.371
Not involved in exercise	16 (59.2)	20 (40)	
Light	1 (3.7)	2 (4)	
Moderate	4 (14.8)	15 (30)	
Heavy	6 (22.2)	13 (26)	
Type of sport			0.339
Not involved in sports	21 (77.7)	45 (90)	
Moderately strenuous sports	5 (18.5)	4 (8)	
Strenuous/ heavy sports	1 (3.7)	1 (2)	

p values were generated with Pearson Chi-square test; Significance considered at $p<0.05$

mechanical integrity which in turn promotes not only disc herniation but also disc degeneration as well [18,19].

Observed BMI of our study population was 23.8±3.8 kg/m^2 where the majority were in the overweight category. Francesco S. Violante *et al* (2004) [20] reported nearly similar mean BMI levels (23.4± 3.8 kg/m^2) in 65 patients with LDH supporting the present findings. Studies reported that obesity and over weight as risk factors for disc herniation [21], however, the exact mechanism is yet unknown. It is assumed that being overweight or obese can increase the compressive loading of the discs causing alterations in the biomechanics of the intervertebral disc thus, leading to disc herniation [21].

A significant association between smoking and disc herniation was not sought in the present study. However, contradictory findings have been reported in the published literature. Studies have reported that smoking in past years was associated with an increased risk of LDH [22]. A case-control study conducted in Pennisylvania with lumbar and cervical disc disease patients (n=205) revealed that smoking history (current and ex-smokers) was significantly high in patients with lumbar disc disease (56 % vs 37 %; $p<0.001$) and cervical disc disease (64.3% vs 37 % $p<0.001$) when compared to controls. Authors further reported the relative

Table 2: Association of IL-6 and hs-CRP levels among chronic and acute groups of patients

Inflammatory marker	Chronic back/neck pain (mean±SE)	Acute back/neck pain (mean±SE)	p value
IL-6 (pg/mL)	197.61±25.11	119.65±24.73	0.043*
hs-CRP (mg/L)	3.50±0.81	8.82±3.14	0.048*

* p values were generated with independent sample t-test; Significance considered at $p<0.05$

risk for smokers was 2.2 folds higher for lumbar disc disease [23]. An *et al* (1994) [23] have further stated that there was a significant association between cigarette smoking and disc disease when compared to current smokers versus non-smokers (lumbar disc disease $p<0.001$ and cervical disc disease $p<0.001$). It has also been revealed the relative risk for current smokers was 3.0 for lumbar disc disease and 3.9 for cervical disc diseases respectively. The authors also reported that continued cigarette smoking can aggravate discogenic or radicular symptoms associated with disc disease. However, consistent findings were reported in a descriptive cross-sectional study conducted among 65 smokers with LDH. ($p= 0.671$). Studies have highlighted that nicotine in cigarettes may cause the narrowing of blood vessels hence impairing the blood flow to the disc tissue causing disc degeneration which may eventually lead to disc herniation. According to assumptions, it is believed that smoking contracts capillary blood vessels and inhibits the diffusion of nutrition to the disc. Further, nicotine is shown to be a significant cell proliferation inhibitor. Therefore, nicotine can affect the cell proliferation in nucleus pulposes and extracellular matrix synthesis and thereby diminish cell proliferation in the NP. Nicotine also inhibits the total collagen production promoting disc herniation [24].

Disc herniation encompasses a spectrum of conventional factors that could directly cause a strain to the vertebral column which in turn leads to complications associated with the disease condition. Some of those conventional factors include heavy physical and mechanical loading, occupational loading, engagement in strenuous sporting activities, awkward bending, vibrations and sitting for long hours [25,26,27]. Therefore, the present study also focused on some selected factors that could promote strain on the vertebral column and can eventually lead to CDH and LDH. However, the present study could not reveal significant associations between; physically demanding nature, duration of occupation, type of exercise, sports involved and CDH or LDH subjects. Francesco S. Violante *et al* (2004) [20] have reported similar findings with involvement in sports. Although the present study did not show a significant association with physically demanding occupations, several other reported studies have reported that physically demanding occupations had a significant role in disc herniation [28,29,30,31]. Manek and MacGregor (2005) stated that heavy physical work is associated with low back pain whereas another study on identical twins also found similar findings [29]. Battie *et al* (1995) [29] have clearly indicated that heavy lifetime occupational and physical loading has an association with disc degeneration in upper lumbar levels whereas sedentary work was associated with mild degeneration ($p=0.006$) causing back pain. However, observations reported by previous studies conducted in monozygotic twins in Finland revealed that there was no significant difference observed in the level of leisure time physical activities with disc herniation [30]. A review has shown that workers with many sedentary activities had higher prevalence rates for pain symptoms and sick leaves due to low back pain [OR=1.46; (95 %

CI=1.18–1.29) for sedentary leisure activities)]. They have also indicated that physical activities in leisure time (either sports or daily physical activities) are not associated with the prevalence rates for low back morbidity [31]. However, other studies had recruited a large sample size, and the limited number of participants in the present study could have been the cause for the current findings. Acute phase proteins such as hs-CRP and CRP elevate in response to inflammation. These markers are regarded as the most sensitive markers of inflammation. Disc herniation induces an inflammatory response around the nerve roots which causes the symptoms such as pain in CDH and LDH [8]. The present study has found significantly elevated IL-6 levels in patients with chronic neck or back pain. Similar findings were reported in previously published data indicating higher levels of pro-inflammatory markers such as ILs, MMP, and prostaglandins [31,32]. Although we have not considered the pain score or nature of pain in this study, the reported studies have also shown increased levels of inflammatory markers in patients with disc degeneration and increased inflammatory markers in higher pain scores [31]. Interestingly, a strong association between pain and hs-CRP levels in patients with acute sciatic pain, reported by Strümmer *et al* (2005) which is compatible with the present findings. However, they were unable to find a relationship between hs-CRP and chronic lower back pain [10]. A notable source of support for our findings was reported by Ackerman and Zhang (2006) where elevation in hs-CRP levels by percentages of 0 %, 20 %, 80 % and 73 % were observed in patients suffering from lumbar disc protrusion, prolapse, extrusion and sequestered types of herniation respectively [33].

In contrast to our findings, a study has highlighted normal hs-CRP values (1.1 mg/L) in patients with lower back pain or radiculopathy due to LDH, spinal stenosis and facet syndrome [9]. Similarly, another group of investigators also argued that hs-CRP could not provide enough evidence to support the relationship between hs-CRP and acute and chronic low back pain [34]. Evidence suggests that prolapsed or degenerated discs can induce the expression of macrophages which release

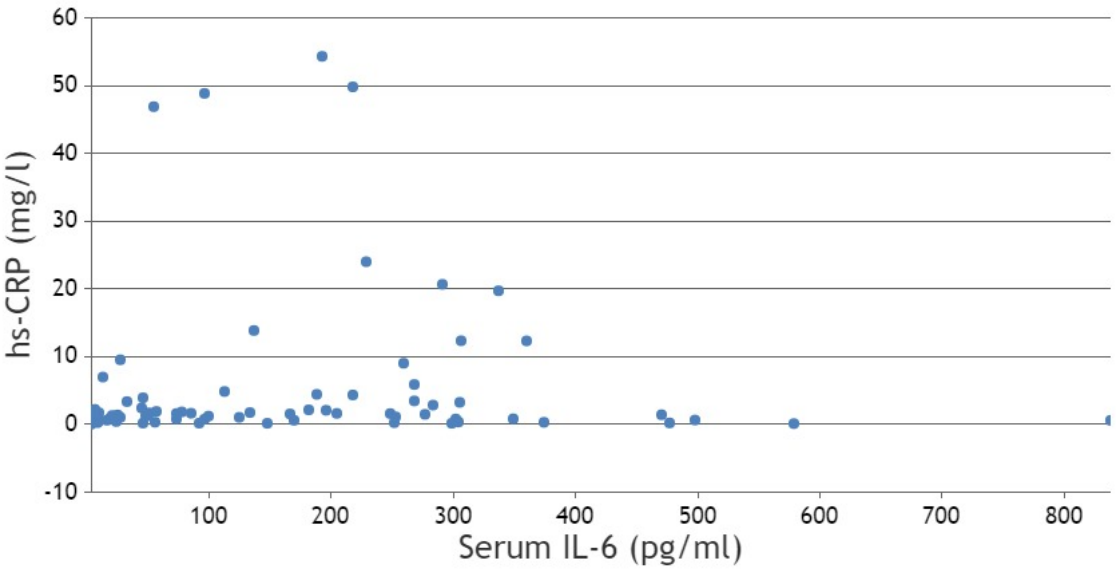

Figure 1: Correlation between IL-6 levels and hs-CRP levels of patients with disc herniation

cytokines, especially IL-6 that trigger the secretion of CRP [8]. As hs-CRP can be elevated in other inflammatory and cardiac diseases we have excluded patients with acute or chronic infection/inflammation and abnormal electrocardiogram findings (ECG is a pre-requite for the surgical procedure). Although evidence suggests that prolapsed or degenerated discs can induce the expression of macrophages which release cytokines, especially IL-6 that trigger the secretion of CRP, we have not obtained a significant correlation between serum IL-6 and hs-CRP levels which needs to be evaluated further. However, similar insights were reported in a study carried out on male athletes validating the present results [35,36].

Conclusion

The absence of a substantial link between IL-6, hs-CRP, CDH, or LDH indicates that these inflammatory biomarkers may not directly influence the development of CDH or LDH. However, increased IL-6 levels in patients with chronic pain indicate a significant association between IL-6 and chronic pain status. In contrast, elevated hs-CRP levels in acute pain patients suggest a distinct inflammatory response specific to acute pain, as opposed to chronic pain. Occupation, exercise and sports demonstrated no significant association with CDH or LDH. Studies are needed to assess the benefit of novel anti-inflammatory treatment in acute pain associated with disc herniation considering the high hs-CRP level observed in the group.

Conflicts of Interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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