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An exploratory cohort study of serum estradiol, testosterone, osteoprotegerin, interleukin-6, calcium, and magnesium as potential biomarkers of cervical spondylosis

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Abstract

Background Exploration of biomarkers for debilitating diseases such as cervical spondylosis is important to revolutionize clinical diagnosis and management of such conditions. The study aimed to determine the correlation between neck pain and disability and serum levels of interleukin-6 (IL-6), osteoprotegerin (OPG), estradiol (E2), testosterone (TES), calcium (Ca), and magnesium (Mg) among individuals with symptomatic cervical spondylosis.

Methods This study was a cohort design. The participants were new referrals to two Nigerian physical therapy clinics. Participants' neck pain intensity (PI), neck disability index (NDI), IL-6, OPG, E2, TES, Ca, and Mg were measured at baseline and after 13 weeks of follow-up. Data were analyzed using descriptive statistics, independent samples *t* test, Pearson's correlation, and multiple linear regression.

Results Forty individuals aged 52.40 ± 8.60 years participated in the study. Women had significantly higher levels of IL-6 ($t = -2.392$, $p = 0.026$), OPG ($t = -3.235$, $p = 0.005$), E2 ($t = -6.841$, $p = 0.001$), but lower TES ($t = 17.776$, $p = 0.001$). There were no significant sex differences in PI and NDI. There were significant correlations between PI and OPG ($r = 0.385$, $p < 0.001$), NDI and OPG ($r = 0.402$, $p < 0.001$), and IL-6 ($r = 0.235$, $p = 0.036$). Significant predictors of PI were OPG ($\beta = 0.442$, $p < 0.001$) and E2 ($\beta = -0.285$, $p = 0.011$), and NDI were OPG ($\beta = 0.453$, $p < 0.001$), E2 ($\beta = -0.292$, $p = 0.005$), and IL-6 ($\beta = 0.225$, $p = 0.024$).

Conclusion High serum levels of IL-6 and OPG were associated with cervical spondylosis severity. However, high serum levels of E2 and TES correlated with lesser severity. Moreover, TES inversely correlated with the proinflammatory cytokines.

Keywords Bone remodeling, Cytokines, Disability evaluation, Intervertebral disc degeneration, Neck pain, Physical therapy, Sex hormones

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Introduction

Research into the biomolecular basis of diseases is gaining interest in physical therapy. Identifying biomarkers associated with the pathogenesis of common diseases will help in developing prophylaxis and improving interventions for such diseases. It will also help explain the biomolecular mechanism of action of physical therapy modalities of known efficacy. A biomarker is a measurable characteristic whose values indicate normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. Recent integrative approaches to chronic pain management focus on neuroinflammation and the roles of chemical mediators such as osteoprotegerin (OPG) and interleukins [2], yet there is a paucity of studies on the role of these biomarkers in prevalent musculoskeletal conditions such as cervical spondylosis [3].

Cervical spondylosis is a common age-related degenerative disease leading to significant neck pain, stiffness, discomfort, disability, and economic burden and it hampers the quality of life among adults globally [4]. Characteristically, cervical spondylosis can begin with cervical facet joint arthritis and a feature of gradual degenerative cervical disc changes, which can lead to the formation of osteophytes around the edges of the vertebrae body [5]. Severe cases may lead to axial joint dysfunction due to ligament, articular cartilage, and facet joint involvement as well as compression of a nerve root or spinal cord causing cervical radiculopathy or myelopathy [5, 6].

Cervical spondylosis contributes significantly to *years lived with disability* (YLD), and 85% of individuals, 60 years and older, show evidence of cervical spondylosis on radiographic imaging [7]. The prevalence of cervical spondylosis in southwestern Nigeria was 10.7% [8]. A sedentary lifestyle, smoking, use of technological devices involving prolonged neck bending (computer and phones), manual handling jobs, menopause, older age, and its associated biophysical decline predisposes individuals to cervical spondylosis [9, 10]. Therefore, countries experiencing increased longevity and civilization are faced with an increased incidence of symptomatic cervical spondylosis and its burden. For instance, the UK spends approximately 681.6 million pounds on residual disability and other complications from cervical spondylosis despite the initial cost of primary interventions [11]. Some of these cost goes into physical therapy in which modalities and procedures such as transcutaneous electrical nerve stimulation, infrared therapy, and cervical traction have been found promising.

Based on the literature, the following biomarkers may correlate with cervical spondylosis severity: interleukin-6 (IL-6) [12, 13], OPG [14], sex hormones (estradiol [E2] and testosterone [TES]), calcium (Ca), and magnesium (Mg) [15–21]. Henceforth, in this study, the term biomarkers represent IL-6, OPG, E2, TES, Ca, and Mg. Briefly, OPG and IL-6 are pro-inflammatory cytokines. OPG is a soluble member of the tumor necrosis factor receptor superfamily with pleiotropic effects on bone metabolism and endocrine function [22]. It can downregulate osteoclastogenesis by regulating nuclear transcription factor kappa B (NFKB) [23]. However, IL-6 can inhibit OPG and facilitate receptor activators of nuclear factor kappa B ligand (RANKL) mRNA activity, thus disrupting the RANKL/OPG ratio and resulting in higher bone damage [24]. Nonetheless, TES and E2 moderate osteoclastogenesis by regulating OPG and IL-6 [25–27]. The complex interaction between the inflammatory cytokines and sex hormones affects the serum levels of Ca and Mg via their osteoclastic activities [27, 28]. Figure 1 is a theoretical framework for the correlation between the biomarkers under study, neck pain, and disability.

This study aimed to explore the correlation between serum levels of IL-6, OPG, E2, TES, Ca, and Mg with the pain intensity (PI) and neck disability index (NDI) and to identify biomarkers and sociodemographic variables that could predict PI and NDI among the cohort. The study hypothesized that there would be no significant (a) correlation between the biomarkers, PI, and NDI, and (b) association between the biomarkers, sociodemographic factors, and each of PI and NDI.

Methods

Study design

This paper is a secondary analysis of a cohort study involving forty gender-matched participants who were diagnosed with cervical spondylosis and referred for physical therapy follow-up. The protocol was approved by the Health Research and Ethics Committee of the Health Research and Ethics Committee of the National Orthopaedic Hospital Enugu (NOHE), Nigeria (IRB/HEC Protocol No: S.313/IV/985). Each participant signed a written informed consent before study entry. The study was conducted following the guidelines of the revised Declaration of Helsinki 2013 and reported in adherence to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist for cohort studies [29].

Cohort description

The cohort were participants of a cross-over cohort study, designed to assess the effect of biomarkers, infrared radiation, and cervical traction therapies on neck pain intensity and disability among people with cervical spondylosis [30, 31]. The study involved adult male and female patients ($n=20$, each), aged between 30 and

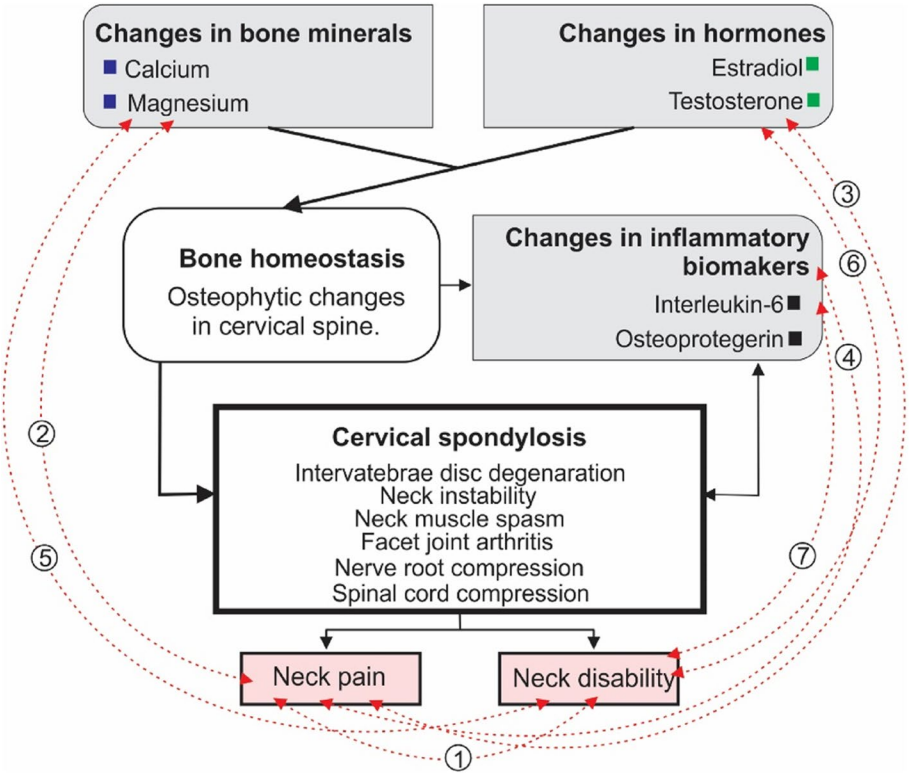

Fig. 1 Theoretical interactions between the biomarkers and primary outcomes. Models 1–7: correlation between neck pain and (1) neck disability, (2) serum calcium and magnesium, (3) serum estradiol and testosterone, (4) serum osteoprotegerin and interleukin-6; the correlation between neck disability and (5) serum calcium and magnesium, (6) serum estradiol and testosterone, and (7) serum osteoprotegerin and interleukin-6

64 years, who were diagnosed with cervical spondylosis with radiculopathy and referred for physical therapy in NOHE and Saint Mary Hospital Enugu (SMHE), Southeast Nigeria. Cervical spondylosis was diagnosed by the consultant orthopedic doctors at NOHE and SMHE using the medical history such as neck pain characteristics and duration, physical examination, and the presence of symptomatic degenerative changes in a magnetic resonance image of the cervical spine [30]. All the patients with positive diagnoses were informed about the study, and patients that provided signed informed consent notes and passed the inclusion criteria were recruited into the study. The study was conducted at the Department of Physiotherapy in both hospitals between 31 March 2016 and 7 March 2018.

Participant eligibility criteria

Participants were included in the study if they were aged between 30 and 65 years, diagnosed with cervical spondylosis, and had at least 1-month history of neck pain with radiculopathy due to cervical spondylosis. Pregnant women, patients with low bone density, ankylosing spondylitis, rheumatoid arthritis, kyphosis, scoliosis, cervical instability, tumor, trauma or fracture, severe hypertension, diabetes, active infection, and any other systemic diseases were excluded.

Sample size determination

A post hoc sample size was calculated using a moderate effect size of 0.25, with an alpha error probability of 0.05, and a power of 0.95. The output showed that 74 samples would have ample power for a fixed model multiple linear regression (G*Power 3.1.9.4 software). However, the pooled data (baseline and follow-up) were 80 samples used for the final analysis.

Sampling and bias

To avoid sampling bias, the authors conducted purposive simultaneous participant recruitment and data collection at NOHE and SMHE [32]. Though we aimed to counterbalance gender in our recruitment, there were more women participants, and we could only achieve a gender-matched cohort when four eligible women from SMHE declined participation for personal reasons.

Variables

Participants' sociodemographic variables were sex (women = 0, men = 1), age (years), and duration since pain onset (months), extracted from the hospital records. Physical measures obtained were weight (kg), height (cm), and body mass index (BMI = height squared/weight [m^2/kg]). Primary outcomes were PI (0 to 10 score) and NDI (0 to 50 score). Secondary outcomes were serum concentration of bone minerals (Ca2 and Mg [mmol/L]), biomarkers of inflammation (OPG and IL-6 [pg/ml]), and hormones (E2 [pg/ml] and TES [ng/ml]). Apart from sex (dichotomous variable), all other variables were continuous/scale.

Procedures and instruments

All the outcomes were measured at baseline and during the 13-week follow-up.

Physical measures

Participants' weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a standard BMI apparatus (RGZ-120, made in China; weight/[height]^2 = BMI) and protocol [33].

Subjective measures

The Numeric Pain Rating Scale (NPRS) was used to measure the participants' PI on an 11-point scale (0–no pain to 10–worst pain imaginable). NPRS is a reliable, valid, and responsive measure of PI among individuals with neck pain [34]. The NDI was used to estimate the participant's neck disability level. The NDI comprised ten items: (seven) related to the activity of daily living, (two) related to pain, and (one) related to concentration. Each item is scored from 0 to 5, the total expected score is 0 to 50; higher scores indicate greater disability. The NDI has good psychometric properties (validity and reliability) in patients with neck pain [34].

Laboratory analysis

In anticipation of their potential impacts on the biomarkers, participants were instructed to refrain from the consumption of any drug, caffeine, alcohol, and exercise for at least 48 h before the data collection [35]. Moreover, serum samples for Ca and Mg were collected under overnight fasting conditions because they can be easily affected by diet [36]. The serum concentrations of the biomarkers were analyzed using participants' 5 mil blood samples, drawn through an antecubital venepuncture between 8:00 AM and 10:00 AM by a phlebotomist [35]. Each participant's sample was shared into three different sample bottles appropriate for the tests. All samples were correctly labeled and transported in a cold box from NOHE and SMHE to the Spectrum Biomedical Laboratory (SBL), Enugu, Nigeria (about 10-min drive). Assays were performed in triplicate of thawed samples, and the median scores were reported.

The *ethylenediamine tetraacetic acid* (EDTA) bottles (Caremax Co., Ltd; Made in China©) were used to collect the sample for serum OPG and IL-6 analyses. The blood samples were centrifuged using a bucket centrifuge (Jenalab-model 800D; made in England) at 3000 rpm for 10 min at 4° C, and the supernatant (serum) was collected using a micropipette and stored frozen at − 20° C until they were required for analysis. The laboratory analysis was completed using a commercial human *enzyme-linked immunosorbent assay* (ELISA) kit (BIOTANG; Made in the USA©). The absorbance was read at 450 nm using a microwell plate reader (Diagnostic Automation-DAR 800; Made in the USA©). The laboratory reference range of IL-6 for healthy adults was 0 to 43.5 pg/ml.

The serum Ca and Mg were analyzed from blood samples collected from the antecubital vein without the tourniquet. The sample was discharged into a lithium heparin bottle and transported immediately to the laboratory; the fresh sample was centrifuged at 3000 rpm. The serum was harvested and analyzed with an automated chemistry analyzer (Roche Diagnostics Ltd., Model: Cobas c 111; Made in Germany©). The laboratory reference ranges for adult human serum Mg were 0.66 mmol/L to 1.07 mmol/L, and serum Ca was 2.2 to 2.7 mmol/L.

The serum E2 and TES (ng/ml) were analyzed from participants' blood samples, collected, and allowed to coagulate in a plain sample bottle for 20 min, and the supernatant was collected using a micropipette and analyzed with an automated immunology analyzer (Roche Diagnostics Ltd., Model: Cobas e 411; Made in Germany©). The laboratory reference ranges for E2 were 30 to 400 pg/mL for premenopausal women and 10 to 50 pg/mL for men. Reference values for TES were 2.50 to 9.50 ng/mL for adult males and 0.10 to 0.90 ng/mL for adult females.

Statistical analysis

The data collected from the study were analyzed with SPSS 26 software (SPSS, Chicago, IL, USA). Baseline data characteristics were analyzed using mean ± standard deviation. The dataset was assessed and fixed for assumptions of parametric statistics: independent samples *t* test, Pearson's correlation, and multiple linear regression [37]. There were issues of missing variables, univariate and multivariate outliers, normality, linearity, or multicollinearity. Therefore, sex differences in participants' baseline characteristics were determined using an independent samples *t* test. For the correlation and regression analyses, the baseline and post-follow-up data were pooled and analyzed. The pooled data met all five assumptions

of comparability: same participants, sampling, instrument, outcomes, and context [38]. Pearson's correlation coefficient was used to analyze the correlation between the biomarkers, PI, and NDI. Multiple linear regressions were completed to determine any of the biomarkers (OPG, IL-6, E2, TES, Ca2, and Mg), sociodemographic and anthropometric variables that could significantly predict PI and NDI.

Results

The participants were 40 individuals (20 men and 20 women) aged (mean ± SD) 52.40 ± 8.60 years and presented with moderate neck pain (PI = 6.90 ± 2.20) and disability (NDI = 21.40 ± 8.84) and medical history of 24.66 ± 27.00 months. There were significant sex differences ($p < 0.05$) in height, BMI, OPG, IL-6, E2, and TES among the participants. The males were taller and had more TES, while females were heavier relative to their heights, and secreted more E2, OPG, and IL-6. Table 1 shows participants' sociodemographic, anthropometric, and clinical characteristics. While the baseline data ($n = 40$) was used to compute the sociodemographic panel (Table 1), pooled data (baseline plus the 13-week follow-up, $n = 80$) was used for correlation and regression analyses (Fig. 2).

Pearson's correlation test (Table 2) showed a strong positive correlation between PI and NDI ($r = 0.896$, $p < 0.001$). Serum OPG correlated with PI ($r = 0.385$, $p < 0.001$) and NDI ($r = 0.402$, $p < 0.001$), while IL-6 correlated with NDI ($r = 0.235$, $p = 0.036$) only. There was no significant correlation between the rest of the biomarkers and the measures of cervical spondylosis severity. However, there were significant negative correlations between IL-6 and TES ($r = -0.295$, $p = 0.008$), and Ca ($r = -0.239$, $p = 0.033$); OPG and TES ($r = -0.451$, $p = < 0.001$); and E2 and TES ($r = -0.701$, $p < 0.001$). There was a positive correlation between OPG and E2 ($r = 0.241$, $p = 0.032$). Due to the hormonal sex differences observed in Table 1, there was a negative correlation between TES (male hormone) and each of E2, OPG, and IL-6 (perceived to be higher in females).

Multiple linear regression models were completed to determine sets of biomarkers, sociodemographic, and anthropometric characteristics that could significantly predict PI and NDI, respectively (Table 3). The forward stepwise approach showed that only OPG ($\beta = 0.450$, $p < 0.001$) and E2 ($\beta = -0.271$, $p = 0.011$) could significantly predict the PI. The model was well fit (F [2, 77] = 10.675, $p < 0.001$). However, only 20% of the total variance could be explained by the model (adjusted $R^2 = 0.20$). The neck disability index could be significantly predicted by OPG ($\beta = 0.453$, $p < 0.001$), E2 ($\beta = -0.292$, $p = 0.005$), and ($\beta = 0.225$, $p = 0.024$). The model was well fit (F [3, 76] = 9.958, $p < 0.001$), and 25% of the total variance was explained (adjusted $R^2 = 0.25$).

Discussion

Formerly, cervical spondylosis defined the processes of cervical disc degeneration; however, the term has been broadened to incorporate vertebral osteophytic changes, osteoarthritis of the Luschka and facet joints, and inflammatory reactions characterized by

Table 1 Sex differences in participants' baseline characteristics

Variables	Total ($n = 40$) Mean ± S.D	Male ($n = 20$) Mean ± S.D	Female ($n = 20$) Mean ± S.D	Mean difference	*t* test	*p* value
Age (years)	52.40 ± 8.60	54.26 ± 8.26	50.55 ± 8.75	3.70	1.376	0.177
Weight (kg)	81.85 ± 10.47	84.22 ± 9.89	79.47 ± 10.47	4.76	1.457	0.162
Height (cm)	165.73 ± 12.84	174.05 ± 10.93	157.40 ± 8.56	16.65	5.365	0.001*
BMI (m^2/kg)	30.06 ± 4.83	27.88 ± 3.03	32.24 ± 5.37	4.36	−3.160	0.003*
Duration (months)	24.66 ± 27.00	23.70 ± 28.26	25.63 ± 26.38	−1.93	−0.223	0.829
PI (NPRS)	6.90 ± 2.20	6.85 ± 2.37	7.33 ± 2.23	−0.48	−0.613	0.541
NDI	21.40 ± 8.84	19.90 ± 7.91	24.00 ± 10.31	−4.10	−1.333	0.209
OPG (pg/ml)	53.76 ± 12.04	49.13 ± 9.72	60.85 ± 11.71	−11.72	−3.235	0.005*
IL-6 (pg/ml)	9.14 ± 3.81	7.78 ± 3.87	10.51 ± 3.31	−2.73	−2.392	0.026*
E2 (pg/ml)	45.25 ± 50.32	9.92 ± 7.49	84.44 ± 48.49	−74.52	−6.841	0.001*
TES (ng/ml)	2.45 ± 2.42	4.72 ± 1.01	0.06 ± 0.04	4.66	17.776	0.001*
Ca (mmol/L)	2.63 ± 0.29	2.63 ± 0.27	2.61 ± 0.32	0.02	0.155	0.876
Mg (mmol/L)	0.85 ± 0.20	0.83 ± 0.21	0.92 ± 0.19	−0.10	−1.435	0.167

PI Pain intensity, measured with the numeric rating scale (NPRS), *NDI* Neck disability index, *OPG* Osteoprotegerin, *IL-6* Interleukin-6, *E2* Estradiol, *TES* Testosterone, Ca^{2+} Serum calcium, Mg^{2+} Serum magnesium

* *t* test was significant at $p < 0.05$ level (2-tailed). Bootstrap *t* test (X1000) and normal *t* test yielded similar results

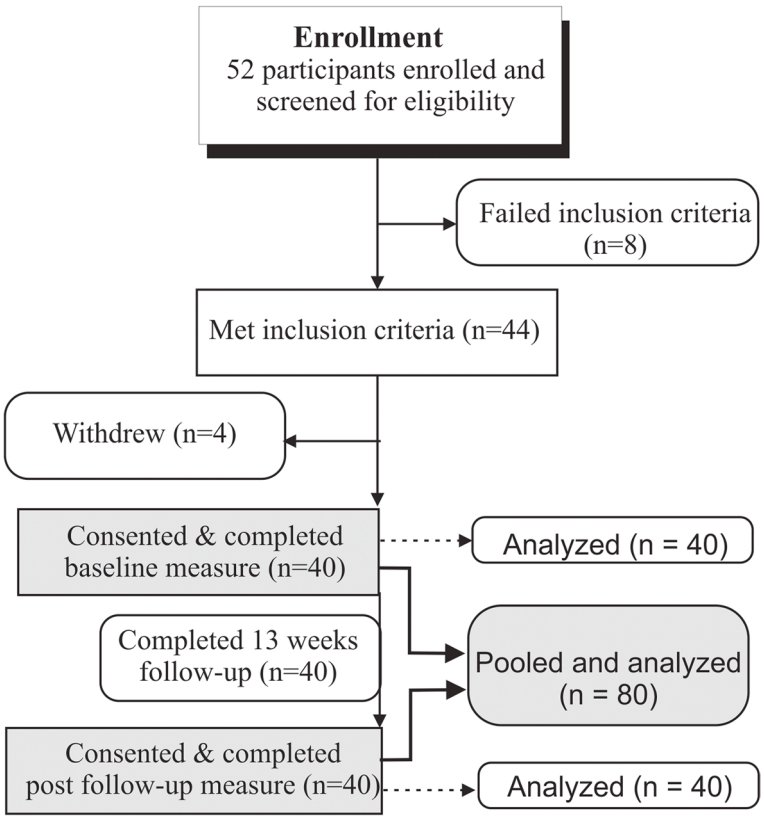

Fig. 2 The study flowchart

Table 2 Correlation between PI, NDI, and the biomarkers ($n = 80$)

Parameters		NDI	OPG	IL-6	E2	TES	Ca	Mg
PI	*r* statistics	0.896	0.385	0.190	−0.163	−0.032	0.026	−0.099
	p value	< 0.001*	< 0.001*	0.091	0.149	0.781	0.816	0.384
NDI	*r* statistics		0.402	0.235	−0.162	−0.145	−0.025	−0.114
	p value		< 0.001*	0.036*	0.151	0.201	0.827	0.315
OPG	*r* statistics			0.084	0.241	−0.451	−0.024	0.057
	p value			0.461	0.032*	< 0.001*	0.830	0.614
IL-6	*r* statistics				0.096	−0.295	−0.239	−0.088
	p value				0.399	0.008*	0.033*	0.436
E2	*r* statistics					−0.701	0.012	0.110
	p value					< 0.001*	0.916	0.330
TES	*r* statistics						0.089	−0.035
	p value						0.431	0.756
Ca	*r* statistics							−0.129
	p value							0.255

PI Pain intensity, measured with the numeric pain rating scale (NPRS), *NDI* Neck disability index, *OPG* Osteoprotegerin, *IL-6* Interleukin-6, *E2* Estradiol, *TES* Testosterone, *Ca* Serum calcium, *Mg* Serum magnesium

* Pearson correlation (*r*) was significant at $p < 0.05$ level (2-tailed)

Table 3 Forward stepwise multiple linear regression showing (biomarkers) predictors of neck pain and disability

(Model) outcome Predictor(s)	Regression coefficient (B)	Standardized coefficient (β)	Partial correlation	*p* value	Tolerance
(1) Pain intensity					
Constant	−1.236			0.369	
Osteoprotegerin	0.121	0.450	0.442	< 0.001*	0.942
Estradiol	−0.017	−0.271	−0.285	0.011*	0.942
(2) Neck disability					
(Constant)	−7.975			0.084	
Osteoprotegerin	0.372	0.453	0.460	< 0.001*	0.938
Estradiol	−0.057	−0.292	−0.317	0.005*	0.936
Interleukin-6	0.685	0.225	0.255	0.024*	0.987

Model summaries

Model 1: $R = 0.47$; adjusted $R^2 = 0.20$; $F(2, 77) = 10.675$, $p < 0.001$

Model 2: $R = 0.53$; adjusted $R^2 = 0.25$; $F(3, 76) = 9.958$, $p < 0.001$

* $p < 0.05$ shows a significant contribution. Predictors entered into the model were age, sex, BMI, height, pain duration, osteoprotegerin, interleukin-6, estradiol, testosterone, calcium, and magnesium

debilitating neck pain and disability [5]. Studies have identified lifestyle, habitual and occupational posture, menopause, and age-related degenerative changes among the predisposing factors of cervical spondylosis [6, 9, 10]. Although the biomolecular mechanisms of these degenerative changes and the inflammatory responses have not been fully understood, the by-products of this degenerative process such as osteophytes and ruptured disc materials may compress neural structure in their canals resulting in cervical radiculopathy, myelopathy, and axial neck pain which are the clinical syndromes of cervical spondylosis [39].

There are prospects for biomarkers in advancing the diagnosis and prognosis of spinal diseases [3, 40]. In this study, serum levels of estradiol, testosterone, osteoprotegerin, interleukin-6, calcium, and magnesium were investigated as potential biomarkers of cervical spondylosis severity. The most common complaints of people with cervical spondylosis are neck pain and disability [30]; therefore, we used NPRS and NDI to estimate the disease severity among the cohort. While older pain theories such as the *pain gait theory* informed structural pathology-based models, recent theories gave insights into the inflammatory basis of spinogenic pain transmission and remission via biomolecular responses [35, 41]. Studies have shown a potential link between cervical degenerative disc diseases and bloodborne mediators of inflammation such as IL-6 [12, 13] and OPG [14]. Although there is a paucity of research that directly linked biomarkers of bone turnover such as sex hormones (E2 and TES), Ca, and Mg with cervical spondylosis [17, 19], some studies have reported an association between bone turnover and cervical spondylosis [16, 18, 21, 42]. Therefore, the conceptual framework in Fig. 1 is plausible. Khan et al. [3] stated that articulating diagnostic and prognostic biomarkers for spinal diseases will be of great clinical relevance.

We found a significant association between OPG and E2 with pain intensity and OPG, E2, and IL-6 with neck disability. However, there was no significant association between other biomarkers, sociodemographic, and anthropometric variables with neck pain intensity and disability via stepwise multiple linear regression. Weber et al. [43] found that serum IL-6 was significantly higher in patients with degenerative disc disease and spinal stenosis than in their counterparts with disc herniation only. Similar to the present study, Du et al. [12] reported that higher IL-6 and OPG were significant predictors of higher pain intensity and disability index. Previous studies have reported that the proinflammatory cytokines (IL-6 and OPG) were mediators of nociception in disc degeneration processes [3, 44]. Apart from the pain-mediating activities of the cytokines, OPG helps to reduce systemic inflammatory bone degeneration and osteoporosis via the inhibition of osteoclastogenesis [28]. The interplay between OPG, E2, and serum Ca2 and Mg improves bone mineral density [45]. Since, osteoporosis has a negative impact on cervical spondylosis [15, 16, 18, 21, 42, 46], higher serum levels of IL-6 might signify a worsening inflammatory process, which may necessitate higher OPG secretion as a protective response.

Pearson's correlation test showed a strong positive correlation between neck pain intensity and disability index, this outcome is expected as the convergent criterion validity of NPRS and NDI is well known [34]. While OPG correlated with neck pain intensity and disability, IL-6 correlated with neck disability only. There was no significant correlation between the rest of the

biomarkers and the measure of cervical spondylosis severity. This outcome agrees with the finding of [12] who reported a positive correlation between IL-6 levels and symptom severity, in a rat model of degenerative cervical myelopathy. As already established in medical research, the present study reported sex differences in E2 and TES levels, such that women had higher E2 and lower TES and vice versa. There was a significant inverse relationship between E2 and TES with PI and NDI. Testosterone levels had a negative correlation with proinflammatory cytokines (IL-6 and OPG) and measures of cervical spondylosis severity. Conversely, E2 had a positive correlation with the cytokines. This implies that women may have more disease severity. Concurringly, Lv et al. [9] reported that cervical spondylosis prevalence was higher in women than in men (16.51 vs 10.49%). With the moderating roles sex home plays in bone metabolism and inflammation, post-menopausal women might have more cervical spondylosis severity than men of similar age.

While serum Mg had no significant correlation with PI, NDI, and the rest of the biomarkers, Ca had a significant inverse correlation with IL-6 levels. The impact of blood levels of calcium and magnesium and their influence via the parathyroid calcium sensory receptor has been found to indirectly downregulate the IL-6 osteoclastic and inflammatory activities [47]. Contrarily, Omoigui [41] suggested that higher serum calcium could be associated with higher IL-6 levels, bone pain, osteoclastic activities, degeneration, and disability. Zhao et al. [48] reported that serum Ca correlated with the degree of disk degeneration, suggesting that serum Ca be used as an indicator of intervertebral disk degeneration prognosis. Understanding the osteoclastic and osteoblastic activities and their biomarkers is crucial to the comprehension of normal bone turnover and could be implicated in metabolic and degenerative bone diseases.

Study significance and strength

The previous paper [30] reported the efficacy of concurrent infrared and cervical traction for cervical spondylosis, the present paper has shown a correlation between cervical spondylosis and some of the biomarkers. The next paper will explore the mechanism of action of infrared therapy and cervical traction via their effects on the biomarkers. This study highlighted some salient points necessary for conducting a successful laboratory assay of biomarkers, and this resource will be helpful to physiotherapists planning biomarker studies. The major strength of this paper is the pooled baseline and follow-up data which improved the statistical power. However, a segregated baseline and follow-up analysis also yielded similar results. Moreover, the paper has provided basics for future exploration of some of these biomarkers that were lacking in the literature with reference to cervical spondylosis and physical therapy interventions.

Limitation

Although the participants were gender-matched, non-probability sampling techniques are prone to sampling bias which may affect the generalisability of this study.

Conclusion

Higher levels of serum interleukin-6 and osteoprotegerin may predict higher cervical spondylosis severity, measured as neck pain intensity and disability. Increased levels of sex hormones (estradiol and testosterone) were inversely correlated with disease severity. Specifically, increasing endogenous estradiol was a significant predictor of milder disease even when we controlled for age and sex. Moreover, serum testosterone levels were inversely correlated with the proinflammatory cytokines. Although the present study could not establish an association between the serum level of bone minerals (calcium and magnesium) and the severity of the disease, there are indications from the literature that higher serum calcium correlates with cervical spondylosis severity.

Abbreviations

Ca	Calcium
E2	Estradiol
IL-6	Interleukin-6
Mg	Magnesium
NOHE	National Orthopedic Hospital, Enugu
NPRS	Numerical Pain Rating Scale
OPG	Osteoprotegerin
PI	Pain intensity
SMHE	St. Mary's Hospital Enugu
TES	Testosterone

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Authors' contributions

AAI, CIE, GCO, and OKO contributed to the conception of this study. All authors made substantial contributions to the design and acquisition of the data. OKO and OOA performed the statistical analysis. OKO, AI, KMO, and CCA were responsible for drafting the article. AAI, CIE, OOA, and GCO contributed to its critical revision. The authors approved the final manuscript for publication.

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Availability of data and materials
The dataset analyzed during the current study is available at the Zenodo repository https://doi.org/10.5281/zenodo.4337859 [31].

Declarations

Ethics approval and consent to participate
The authors obtained ethical approval to conduct this study from the Health Research and Ethics Committee of the Health Research and Ethics Committee of the National Orthopaedic Hospital Enugu (NOHE), Nigeria (IRB/HEC Protocol No: S.313/IV/985). The objectives, protocols, benefits, and potential harms of the study were clearly explained to the participants. Each participant signed a written informed consent before participating in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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