



Comparison of Whole Spine Sagittal Alignment in Patients with Spinal Disease between EOS Imaging System versus Conventional Whole Spine X-ray

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Purpose: The biplanar whole body imaging system (EOS) is a new tool for measuring the whole body sagittal alignment in a limited space. This tool may affect the sagittal balance of patients compared to conventional whole spine X-ray (WSX). This study aimed to investigate the difference in sagittal alignment between WSX and EOS.

Materials and Methods: We compared the spinal and pelvic sagittal parameters in 80 patients who underwent EOS and WSX within one month between July 2018 and September 2019. The patients were divided based on sagittally balanced and imbalanced groups according to pelvic tilt (PT) >20°, pelvic incidence-lumbar lordosis >10°, C7-sagittal vertical axis (SVA) > 50 mm in WSX.

Results: In the sagittally imbalanced group, compared to WSX, the pelvic parameters demonstrated compensation in EOS with smaller PT (27.4±11.6° vs. 24.9±10.9°, $p=0.003$) and greater sacral slope (SS), and the patients tended to stand more upright with smaller C7-SVA (58.4±17.0 mm vs. 48.9±57.3 mm, $p=0.018$), T1-pelvic angle (TPA), T5-T12, and T2-T12. However, in the sagittally balanced group, these differences were less pronounced only with smaller PT (10.8±6.9° vs. 9.4±4.7°, $p=0.040$), TPA and T2-T12 angle, but with similar SS and C7-SVA ($p>0.05$).

Conclusion: EOS showed a negative SVA shift and lesser PT compared to WSX, especially in patients with sagittal imbalance. When preparing a surgical plan, surgeons should consider these differences between EOS and WSX.

Key Words: EOS, whole spine x-ray, compensatory mechanism, sagittal parameters

INTRODUCTION

Due to the correlation between self-reported pain and disability, accurate assessment of sagittal parameters of patients

with spinal malalignment has become an important factor.¹⁻⁴ Several studies tried to reveal a compensatory mechanism for the reciprocal change interrelationship between spine-pelvis-hip joint and lower extremities.^{5,6} To avoid translation of the gravity line of the body due to progressive disc degeneration and loss of disc height, there is frequent hyperextension of the adjacent segment at the thoracic and cervical levels. Cervical hyperlordosis and thoracic hypokyphosis result from the rebalancing efforts, as described by Dubouset⁷ in his concept of an “efficiency cone.” Patients use the retroversion mechanism at the level of pelvis, and this occasionally translates into extension of the hip joints.⁸⁻¹¹ At the lower extremity level, small lumbar lordosis (LL) is associated with a large pelvic shift and compensatory knee flexion.¹²

The EOS imaging system (EOS imaging, Paris, France), which

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uses an ultrasensitive multiwire proportional chamber detector, allows simultaneous anteroposterior (AP) and lateral 2D imaging of the whole body in a calibrated environment. However, since EOS whole body image is conducted in a narrow space, patients are required to maintain a standing balanced posture during examination (Fig. 1). Unlike in EOS examination, patients stand relatively free under instructions in open space during conventional whole spine X-ray (WSX) evaluation.¹³ Sagittal and spinopelvic parameters may vary significantly depending on the patient's posture even in the same modality of radiographs.¹⁴⁻¹⁶

Before surgical treatments for deformity, patients are usually evaluated with EOS instead of WSX. The sagittal and spinopelvic parameters may differ between EOS and WSX, but we are not aware of any research on this topic. Therefore, this study was conducted to elucidate any sagittal parameter differences between WSX and EOS.

MATERIALS AND METHODS

Study design

We reviewed medical records of 113 patients who had undergone both EOS and WSX at a single institution between July 2018 and September 2019. Since EOS was first introduced to our hospital in 2018, the patients had undergone both WSX and EOS during the transition period. A total of 80 patients who satisfied the following criteria were enrolled in this study: patients aged 18 years or older, those whose examination interval between EOS and WSX was less than 1 month, and those who had not undergone surgery or suffered spinal infection prior to each examination. Exclusion criteria included the follow-

ing: previous history of spine deformity, knee, or hip surgery; major coronal deformity (Cobb angle $\geq 30^\circ$); inability to stand unaided due to severe sagittal imbalance or weakness; traumatic fracture; malignancy; or congenital, neuromuscular, or connective tissue disorders. Furthermore, to demonstrate the sagittal imbalance differences between the two test modalities, we divided the patients into two groups: sagittally balanced group and sagittally imbalanced group. Sagittally imbalanced group was defined as patients who had any of pelvic incidence (PI)-LL $>10^\circ$, C7-sagittal vertical axis (SVA) >5 cm, pelvic tilt (PT) $>20^\circ$ in either EOS or WSX; and the remaining patients were classified into sagittally balanced group. Institutional Review Board approval was received for this study with a waiver of informed consent (No. 2020-0031-001).

Radiologic examination

All eligible patients underwent both EOS and WSX in an upright standing position. In EOS, we followed the manual of other studies. We instructed the patients to place fingertips of both hands on zygomas with the upper arms at an angle of approximately 45° to vertical, and rest in a comfortable position for about 10 seconds before the examination.¹³ For WSX, our study used the Radiographic Measurement Manual introduced by the Scoliosis Research Society; 36-inch whole-spine AP and lateral plain radiographs were collected at a 72-inch distance from the film. The patients stood in a comfortable position with their knees locked, feet at shoulder width apart, looking straight ahead, with their elbows bent, and fists on clavicles.^{13,14}

Data collection

Baseline demographics of age, sex, and body mass index (BMI) were recorded. The spinopelvic balance was evaluated at the

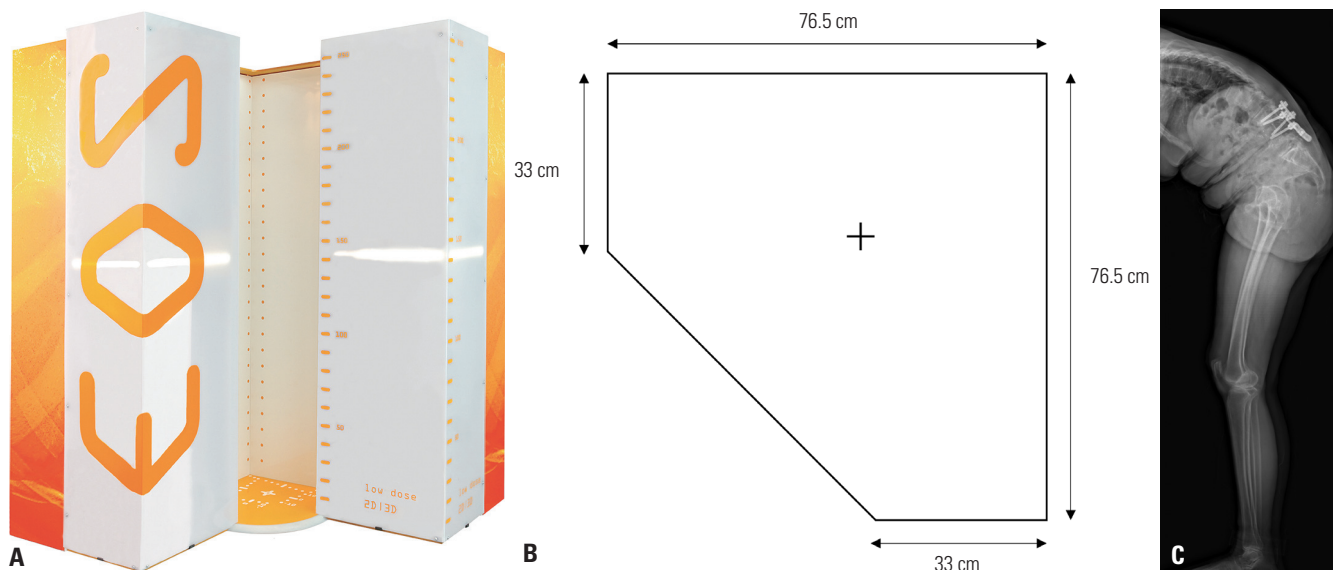


Fig. 1. (A) EOS imaging system (<https://www.eos-imaging.com/professionals/materials>). Perpendicularly placed, vertically moving, co-linked units of X-ray tubes producing very thin collimated X-ray beams collected by unique line detectors with Nobel Prize-winning technology resulted in simultaneously captured biplanar digital images of a patient in a standing, weight-bearing position. (B) A space on which the patient stands. The width and length were about 76.5 cm each. (C) A patient with severe sagittal deformity whose entire body could not be examined with an EOS system.

C7-SVA and T1-pelvic angle (TPA), the angle between the line from the femoral head axis to the center of the T1 vertebra, and the line from the femoral head axis to the middle of the S1 superior endplate, respectively.

The following spinopelvic parameters were measured: PT, PI, sacral slope (SS), LL, PI minus LL, thoracic kyphosis (T2-T5, T5-T12, and T2-T12 Cobb angles), and thoracolumbar kyphosis (T10-L2 Cobb angle). The cervical parameters were C0-C2 angle (occiput to C2 Cobb angle); C2-C7 lordosis (C2-C7 Cobb angle); C2-C7 SVA, the horizontal distance between a plumb line dropped from C2 to the postero-superior corner of C7; and T1 slope, the angle between the superior endplate of T1 and a horizontal reference line.

The lower extremity parameter was the sacrofemoral angle (SFA), the angle formed between the bicoxofemoral axis and the line tangent to the superior endplate of S1, and the line between the bicoxofemoral axis and the femoral axis. Since there

were no WSX measurement below the knees, the knee flexion angle or ankle dorsiflexion angle was not measured. The spinal balance parameter was the sagittal C2 (C7) plumb line, the distance between the plumb lines passing through HA and the plumb lines from the centroids of C2 (C7) vertebral bodies.

Each parameter was measured with imaging software (Centricity Enterprise Web V 3.0; GE Medical Systems, Milwaukee, WI, USA). All measurements were taken independently by two experienced spinal surgeons to assess inter-rater variability. For intra-rater reliability, measurements were made twice at 1-month intervals. The average value was used to minimize intra-rater and inter-rater errors.

Statistical analysis

Inter-rater and intra-rater reliability analysis between the measurers was performed using interclass correlation coefficients. All parameters were measured at values of 0.8 or higher, repre-

Table 1. Demographics and Sagittal Parameters of Study Patients

Demographics	All patients (n=80)	Sagittally balanced patients (n=28)	Sagittally imbalanced patients (n=52)	p value
Age (yr)	63.2±13.3	55.5±14.0	67.3±11.0	0.010*
Sex (male/female)	40/40	20/8	20/32	0.005 *
Weight (kg)	64.8±13.3	68.3±9.0	62.9±12.9	0.051
Height (cm)	160.7±9.9	166.3±8.1	157.8±9.6	0.001*
BMI (kg/m ²)	25.1±4.24	24.7±2.7	25.3±4.9	0.582
Pathology				
Cervical myelopathy	6	5	1	
Degenerative spondylolisthesis	28	8	20	
Herniated lumbar disc	8	2	6	
Lumbar degenerative kyphosis	6	0	6	
Spondylolytic spondylolisthesis	3	0	3	
Lumbar stenosis	29	13	16	
Sagittal parameters				
PI				
WSX	50.2±11.5	42.6±6.4	54.2±11.7	<0.001*
EOS	50.6±11.5	43.2±6.9	54.6±11.6	<0.001*
LL				
WSX	-35.3±14.5	-44±7.9	-30.5±15.1	<0.001*
EOS	-35.5±14.4	-44.3±8.9	-30.8±14.6	<0.001*
PI-LL				
WSX	14.9±18.8	-1.4±6.7	23.7±17.3	<0.001*
EOS	15.1±18.9	-1.1±7.0	24.0±17.3	<0.001*
PT				
WSX	21.6±12.2	10.8±4.7	27.4±10.9	<0.001*
EOS	19.4±11.5	9.4±5.7	24.9±10.2	<0.001*
SS				
WSX	28.6±8.7	32.6±7.0	26.5±8.9	0.002*
EOS	30.8±8.1	33.9±5.9	29.2±8.7	0.014*

BMI, body mass index; WSX, conventional whole spine X-ray; PI, pelvic incidence; LL, lumbar lordosis; PT, pelvic tilt; SS, sacral slope.

Descriptive data are presented as mean±standard deviation. Positive values signify an anterior position of the vertical plumb line with respect to the caudal landmark for translation measurements and kyphosis for angular measurements.

* $p < 0.05$.

senting excellent reliability.¹⁷ To determine EOS and WSX differences, paired t-tests or Wilcoxon test were used on the measured data from all patients depending on the normality of distribution of each parameter. Data analysis of the sagittal imbalance group was performed separately. All statistical analysis was conducted using IBM SPSS Statistics ver. 25.0 (IBM Corp., Armonk, NY, USA), with statistical significance set at $p < 0.05$ throughout.

RESULTS

Patient demographics

Overall, the average age of patients was 63.2 years, and there were 40 male and 40 female patients. The average patient BMI was $25.1 \pm 4.24 \text{ kg/m}^2$. Degenerative spondylolisthesis and spondylotic stenosis were predominant (57 of 80). Fifty-two patients were in the sagittally imbalance group, and 28 patients were in the sagittally balanced group. In the sagittally imbalanced group, male/female sex ratio was higher (20/32) compared that in the sagittally balanced group (20/8), with older age (67.3 years to 55.5 years, $p = 0.010$), lower height and weight, and similar BMI. In both WSX and EOS, the sagittally imbalance group had larger PI, PI-LL, and PT and smaller PT and SS compared to the sagittally balanced group (Table 1).

Comparison of sagittal balance, spinal curvature, spinopelvic parameters, and lower limb parameter between EOS and WSX in sagittally imbalanced group

A number of significant radiological differences between EOS and WSX were observed. C7-SVA was significantly greater in WSX ($58.4 \pm 17.0 \text{ mm}$) than in EOS ($48.9 \pm 57.3 \text{ mm}$, $p = 0.018$). WSX TPA was $26.1 \pm 13.9^\circ$, while that for EOS TPA was $22.9 \pm 12.0^\circ$

Table 2. Comparison of Sagittal Balance, Spinal Curvature, Spinopelvic Parameters, and Lower Limb Parameters between WSX and EOS in Sagittally Imbalanced Patients[†]

	WSX	EOS	p value
Sagittal balance			
C2-C7 SVA (mm)	21±47.2	21.8±15.3	0.644
C2-HA (°)	5.3±6.9	5.3±6.3	0.973
C7-HA (°)	5.4±7.0	4.9±7.2	0.320
C7-SVA (mm)	58.4±17.0	48.9±57.3	0.018*
TPA (°)	26.1±13.9	22.9±12.0	0.002*
Spinal curvature (°)			
T1-slope	21.7±11.1	20.9±10.7	0.352
C0-C2 angle	-30±10.2	-28.1±12.8	0.461
C2-C7 lordosis	-12.6±14.8	-8.2±13.7	0.008*
T2-T5 angle	9.5±15.9	10±9.9	0.456
T5-T12 angle	21±14.4	17.7±14.4	0.001*
T2-T12 angle	26.6±14.6	23.6±14.5	0.010*
T10-L2 angle	11.3±10.6	8.9±16.7	0.133
Spinopelvic parameters (°)			
PI	54.2±8.7	54.6±11.7	0.697
LL	-30.5±17.5	-30.8±15.1	0.851
PI-LL	23.7±9.8	23.9±17.3	0.837
PT	27.4±11.6	24.9±10.9	0.003*
SS	26.5±14.6	29.2±8.9	0.006*
Lower limb parameter (°)			
SFA	150.4±51.5	152.5±23.4	0.519

WSX, conventional whole spine X-ray; SVA, sagittal vertical axis; TPA, T1-pelvic angle; PI, pelvic incidence; LL, lumbar lordosis; PT, pelvic tilt; SS, sacral slope; SFA, sacrofemoral angle.

Descriptive data are presented as mean±standard deviation.

* $p < 0.05$; [†]Sagittally imbalanced patients had one of the following conditions: PI-LL $> 10^\circ$, SVA $> 5 \text{ cm}$, and PT $> 20^\circ$ on WSX. Positive values signify an anterior position of the vertical plumb line with respect to the caudal landmark for translation measurements and kyphosis for angular measurements.

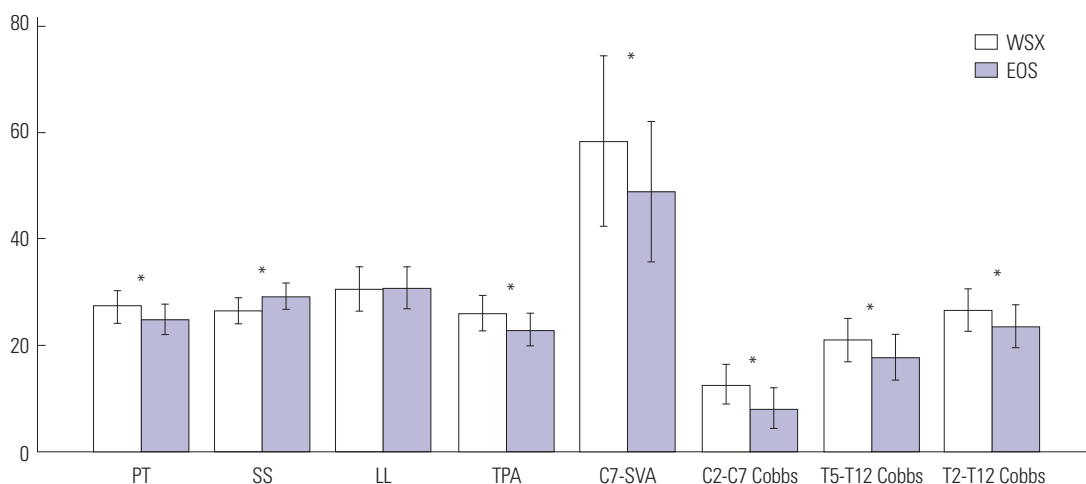


Fig. 2. Comparisons of sagittal parameters between WSX and EOS in patients with sagittal imbalance. *Indicates significant differences in angles between WSX and EOS ($p < 0.05$). For LL and C2-C7 Cobbs, positive values indicate lordosis and negative values indicate kyphosis. WSX, conventional whole spine X-ray; PT, pelvic tilt; SS, sacral slope; LL, lumbar lordosis (L1-S1 Cobbs angle); TPA, T1-pelvic angle; SVA, sagittal vertical axis.

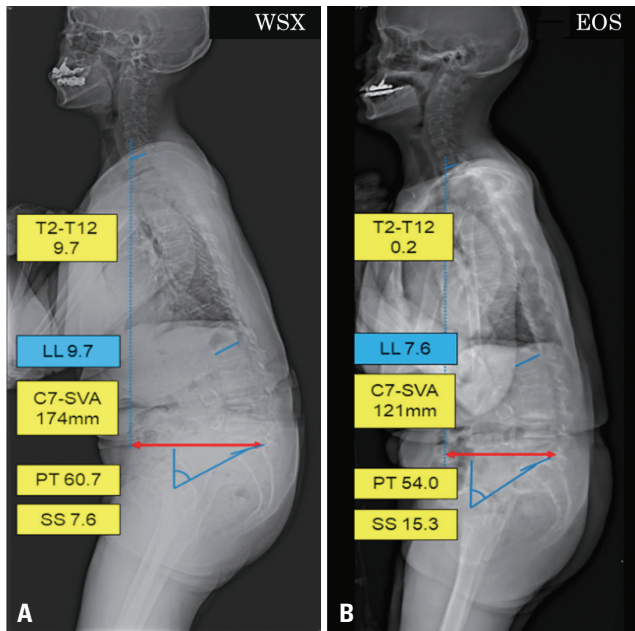


Fig. 3. Typical case of the sagittally imbalanced group. Compared to WSX (A), EOS (B) showed smaller PT (60.7° to 54.0°), C7-SVA (174 to 121 mm), T2-T12 (9.7° to 0.2°), and larger SS (7.6° to 15.3°), indicating a compensation. WSX, conventional whole spine X-ray; PT, pelvic tilt; SS, sacral slope; SVA, sagittal vertical axis.

($p=0.002$). The kyphosis of T2-T12, T5-T12, and the lordosis of C2-C7 were decreased in EOS compared to WSX. The LLs were similar between the two tests, but spinopelvic parameters demonstrated compensation in EOS. EOS PT (24.9±10.9°) was decreased compared to WSX PT (27.4±11.6°, $p=0.003$), and EOS SS (29.2±8.9°) was increased compared to WSX SS (26.5±14.6°, $p=0.006$) (Table 2 and Fig. 2). Typical cases of the sagittally imbalanced patients are illustrated in Fig. 3.

Subgroup comparison of EOS and WSX in sagittally balanced group

In sagittally balanced patients, EOS PT (10.8±6.9° vs. 9.4±4.7°, $p=0.040$), TPA (7.1±9.3° vs. 4.9±4.8°, $p=0.010$), C2-C7 lordosis (-6.3±13.4° vs. -2.4±11.2°, $p=0.033$), and T2-T12 angle (33.9±7.4° vs. 31.1±9°, $p=0.042$) were decreased compared to those of WSX. However, SS, C7-SVA, and T5-T12, which showed differences in the sagittally imbalanced group, did not show differences in EOS and WSX in the sagittally balanced group (Table 3 and Fig. 4).

DISCUSSION

For spinal deformity surgery, knowledge of whole-body alignment, including spine and lower extremities, is essential. This knowledge is the basis for decisions regarding spinal deformity treatment strategy. This knowledge also allows for the prediction of postoperative complications, such as proximal/distal junctional problem, mechanical failure, and poor surgical outcome.^{10,14,18-20}

Table 3. Comparison of Sagittal Balance, Spinal Curvature, Spinopelvic Parameters, and Lower Limb Parameter between WSX and EOS in Sagittally Balanced Patients[†]

	WSX	EOS	<i>p</i> value
Sagittal balance			
C2-C7 SVA (mm) [‡]	21.1±25.1	18.4±9.3	0.244
C2-HA (°)	-0.7±3.2	-0.5±3.3	0.819
C7-HA (°)	-1.9±7.0	-1.6±3.2	0.489
C7-SVA (mm)	0.3±10.2	2.0±32.0	0.763
TPA (°)	7.1±9.3	4.9±4.8	0.010*
Spinal curvature (°)			
T1-slope	18.6±6.0	16.2±9.3	0.159
C0-C2 angle	-27.3±7.1	-34.0±23.8	0.179
C2-C7 lordosis	-6.3±13.4	-2.4±11.2	0.033*
T2-T5 angle	13.2±10.5	12.6±7.2	0.561
T5-T12 angle	22.8±11.3	21.3±9.8	0.241
T2-T12 angle	33.9±7.4	31.1±9.0	0.042*
T10-L2 angle	6.3±8.1	5.2±8.0	0.171
Spinopelvic parameters (°)			
PI	42.6±5.9	43.2±6.4	0.496
LL	-44±7.0	-44.3±7.9	0.719
PI-LL	-1.4±9.9	-1.1±6.7	0.668
PT	10.8±6.9	9.4±4.7	0.040*
SS	32.6±8.9	33.9±7.0	0.158
Lower limb parameter (°)			
SFA	163.8±30.9	162.7±7.0	0.473

WSX, conventional whole spine X-ray; SVA, sagittal vertical axis; TPA, T1-pelvic angle; PI, pelvic incidence; LL, lumbar lordosis; PT, pelvic tilt; SS, sacral slope; SFA, sacrofemoral angle.

Descriptive data are presented as mean±standard deviation.

* $p<0.05$; [†]Sagittally balanced patients had none of the following conditions: PI-LL >10°, SVA >5 cm, and PT >20° on WSX. Positive values signify an anterior position of the vertical plumb line with respect to the caudal landmark for translation measurements and kyphosis for angular measurements; [‡]Data did not follow a normal distribution, and Wilcoxon test was used.

We found that the patient expression of compensatory mechanisms for balance was shown more in EOS than in WSX. Also, sagittally imbalanced patients showed more differences in sagittal parameters compared to sagittally balanced patients. Global balance, TPA, and C7-SVA were better restored in EOS than in WSX. In EOS, patients had more cervical lordosis, less thoracic kyphosis, less PT, and higher SS than in WSX. We believe that using more compensatory mechanisms in EOS resulted in restored TPA and C7-SVA (Fig. 5). However, in sagittally balanced patients, we observed smaller values in EOS only in TPA, PI, C2-C7 angle, and T2-T12 angle; on the other hand, SS and C7-SVA did not show any difference, as the sagittally balanced patients were already well-compensated. TPA, a known independent parameter from pelvic retroversion and knee flexion, differed from C7-SVA. Since the patients in this study did not undergo surgery, in contrast to the patients included in the Protosaltis study, they had alterations in thoracic kyphosis, which is believed to be the reason for the differences in TPA between the two tests.²¹

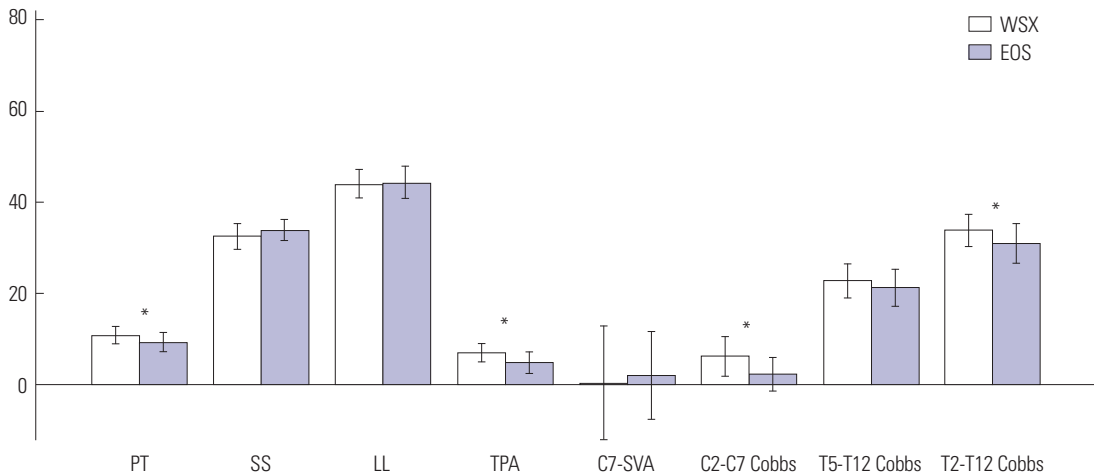


Fig. 4. Comparisons of sagittal parameters between WSX and EOS in patients without sagittal imbalance. *Indicates significant differences in angle between WSX and EOS ($p < 0.05$). For LL and C2-C7 Cobbs, positive values indicate lordosis and negative values indicate kyphosis. WSX, conventional whole spine X-ray; PT, pelvic tilt; SS, sacral slope; LL, lumbar lordosis (L1-S1 Cobbs angle); TPA, T1-pelvic angle; SVA, sagittal vertical axis.

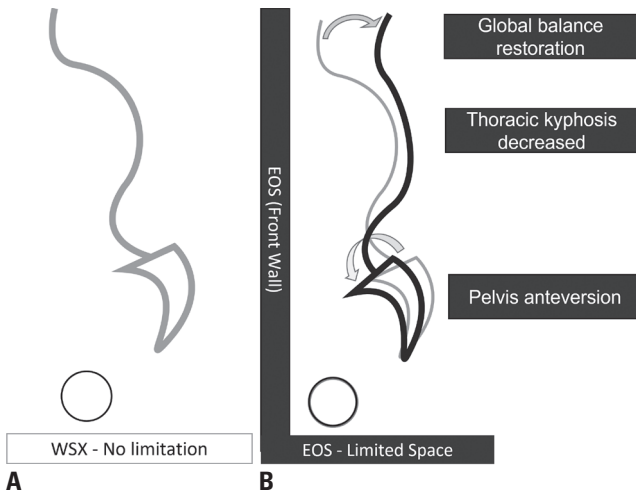


Fig. 5. A figure showing the change of the entire spinal balance in EOS. (A) Directed standing in open space (conventional whole spine X-ray, gray line) vs. (B) Directed standing within limited space (EOS, black line). WSX, conventional whole spine X-ray.

The reason for the differences in sagittal parameters of the two tests is probably the change of patient alignment in limited space and anterior closed wall of the EOS apparatus. While attempting to stand in the limited space of EOS, patients would, consciously or unconsciously, use more compensatory mechanisms to move the gravity line posteriorly. Hey, et al.¹⁵ reported that, compared to directed standing, relaxed standing results in a more kyphotic sagittal profile that is marked by significantly smaller LL and SS and larger PT, thoracic kyphosis, and T1-slope. In our study, compared to the result of Hey, et al.¹⁵, EOS showed a similar radiographical change to directed standing, whereas WSX was similar to natural standing. When patients try to stand upright while undergoing EOS, the sagittal profiles are changed to less kyphotic and shows smaller PT and larger SS, and hips are more extended. In sagittally unbalanced patients, however, the difference between the two tests for PT

and SS compared to the cervico-thoracic parameter are minor, requiring caution in interpretation of the results. To determine the precise difference of PT and SS between EOS and WSX, additional research with larger number of patients or trials including patients who have had thoracolumbar fixation are required.

While there were differences in LL between the two groups in Wong's study, no difference was found in our study between the two groups of EOS and WSX. The reason for the different results in LL is likely due to the differences in study groups; for instance, the healthy population had an average age of 21 years, while patients with spinal disorders had an average age of 63.2 years. Loss of LL is an initial phenomenon in degeneration, not a result of compensation. Patients losing LL due to degenerative lumbar disease experience compensation in other areas.

There could be posture differences due to varying instructions. EOS patients were instructed to place their fingertips on the zygoma, while WSX patients were asked to place them in the clavicular fossa. Differences in the hand position may affect the various parameters of this study. There are debates that SVA or LL may vary depending on the hand position of patients.^{16,22} However, it is difficult to consider the results of this study as a change according to the position of the hand, as Kaneko, et al.²³ reported that, compared to this study, there was no significant difference between the sagittal angles in the posture of raising the hand on the zygoma and raising the hand on the clavicle in EOS.

When planning the surgery, surgeons should consider the fact that the sagittal parameters measured by EOS are affected by the compensatory mechanism, which results from the effort of patients trying to stand upright in a limited space. In addition, when using EOS for post-operative evaluation, the sagittal imbalance may be evaluated as being less severe than the patient's true state. If the patient's sagittal imbalance is too se-

vere, it should be recognized that the EOS scan may not fully reflect the patient's imbalance. To solve these issues, it is essential to establish a precise indication for EOS. Also, further development of biplanar imaging system with open space would be helpful in obtaining accurate sagittal parameters.

The limitations of this study included its retrospective nature, small number of patients, lack of control group, no consideration of the radial X-ray properties of WSX, and the partial measurement of lower leg parameters in WSX, as it does not involve filming below the hip joint. However, we were able to compare SFA in our data, and there was no difference between the two tests. Sagittally imbalanced subgroup patients did not have severe sagittal deformity that requires surgical intervention. Due to the nature of EOS, obtaining the alignment angle from the severely malaligned patients who cannot stand in confined spaces was impossible. Therefore, we excluded these confounder patients from our study. Moreover, this study has no control group who did not have any spinal diseases. In addition, EOS and WSX were performed within 1 month, not simultaneously. This may have caused difficulties in making an accurate comparison due to the changes in patient condition or progression of the disease. Therefore, we excluded the patients with a gap of 1 month or more between EOS and WSX examinations, as well as those who showed substantial disease condition changes in the first month. EOS is a non-radial X-ray system, whereas WSX is a radial X-ray system. As the object moves further from the center of the source, measurement distortion may occur; however, this concern was insufficient in our research. To reduce the amount of distortion, multiple WSX X-rays should be obtained and combined.

In conclusions, sagittal parameters of patients with sagittal imbalance were different according to EOS or WSX. Pelvic anteversion with reduced PT and increased SS was more pronounced in EOS compared to WSX. Less sagittal parameters could show differences according to EOS or WSX in sagittally balanced patients. This difference could be the result of the limited EOS space. Our finding suggests that EOS is a better reflection than WSX of the patient compensatory mechanism in maintaining standing balance.

AUTHOR CONTRIBUTIONS

Conceptualization: Yoon Ha and Kyung Hyun Kim. **Data curation:** Dong Kyu Chin, Keun Su Kim, and Yong Eun Cho. **Formal analysis:** Hyun Jun Jang and Kyung Hyun Kim. **Methodology:** Jeong Yoon Park and Sung Uk Kuh. **Project administration:** Hyun Jun Jang and Dong Kyu Chin. **Visualization:** Hyun Jun Jang and Kyung Hyun Kim. **Writing—original draft:** Hyun Jun Jang and Kyung Hyun Kim. **Writing—review & editing:** Hyun Jun Jang and Kyung Hyun Kim. **Approval of final manuscript:** all authors.

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