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Can Pixel Value Ratio be Used in the Assessment of Ceramic Bone Substitute Incorporation? Observations from a Pilot Study

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Balasubramanian Balakumar[1ABCDEF], Anitha Jasper[2BDE], Roshan S. Livingstone[2CDE], Sangeet Gangadharan[1BEF], Sridhar Gibikote[2AE], Vrisha Madhuri[1ACDEF]

[1] Pediatric Orthopedic Unit, Christian Medical College, Vellore, India
[2] Department of Radiodiagnosis, Christian Medical College, Vellore, India

Author's address: Vrisha Madhuri, Paediatric Orthopaedic Unit, Christian Medical College, Vellore, Tamil Nadu, India – 632004, e-mali: madhuriwalter@cmcvellore.ac.in

Summary

Background: Assessment of bone graft substitute incorporation is critical in the clinical decision making process and requires special investigations. We examined if the pixel value ratio (PVR) obtained in routine follow-up digital radiographs could be used for such assessment.

Material/Methods: Radiographic images were acquired using either computed radiography or flat panel digital radiography systems. The PVR from radiographs of thirty children with ceramic bone substitute grafting were analyzed using the software from the picture archival and communication system (PACS) workstation. Graft incorporation was also assessed using the van Hemert scale. Three independent observers (A, B, C) measured PVRs at two different time points during the first and the last follow-up visits. PVR was compared with the van Hemert scale scores and analyzed using Spearman's rank correlation.

Results: The mean intra-observer reliability was 0.8996, and inter-observer reliabilities were 0.69 (A *vs.* C), 0.78 (A *vs.* B), and 0.85 (B *vs.* C) for the first follow-up visit and 0.74 (A *vs.* C), 0.82 (A *vs.* B), and 0.70 (B *vs.* C) for the last follow-up measurements. Spearman's correlation showed a strong negative association between PVR values and van Hemert scale scores, as the healing process advanced on serial measurements at each follow-up ($r=-0.94$, $n=60$, $z=-7.24$, $p\leq0.0001$). The reliability of the PVR measurements was assessed using an aluminum step wedge and ceramic graft.

Conclusions: PVR is potentially a reliable indicator of bone graft incorporation and can aid in clinical decision making provided standard radiographic techniques are used.

MeSH Keywords: **Bone Density • Bone Substitutes • Radiography**

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Background

Ceramic scaffolds are used extensively as bone graft substitutes in orthopedic surgery. Assessment of their incorporation is essential to clinical decision making. Methods for such assessment include 18F fluoride Positron Emission Tomography – Computed Tomography (PET-CT) [1] and three dimensional CT for spinal inter-body fusion [2], dynamic radiographs [3], scintigraphy [4], histomorphometry [5], magnetic resonance imaging (MRI) [6], dual energy X ray absorptiometry (DXA) [7], and histopathology [8]. However, these techniques have their limitations, and require additional imaging or invasive procedures.

A few studies have reported on the use of the pixel value ratio (PVR) derived from routine digital radiography (DR) for the assessment of mineralization of regenerated bone but not for the assessment of incorporation of ceramic grafts [9–11]. In this study, we planned to measure PVR values for ceramic grafts from computed radiographic (CR) and flat panel DR images using post processing software available on the picture archival and communication system (PACS) and evaluate the reliability of this technique using standard phantoms.

Material and Methods

We prospectively analyzed graft incorporation in thirty children who underwent ceramic bone substitute implantation to fill bone defects. Institutional review board approval and ethical clearance were obtained. The study comprised 30 subjects, 14 girls and 16 boys. All children received commercially available ceramic bone substitute implants (triphasic hydroxyapatite + tricalcium phosphate + calcium silicate [HASi] Biograft, India). These were used for procedures like pelvic osteotomies, calcaneal lengthening osteotomies, ulnar lengthening and angulation osteotomy, subtalar arthrodesis and valgus osteotomy of the proximal femur, treatment of intercondylar fracture of the humerus, segmental bone defects, and cystic benign bone tumors. No additional bone grafts or other bone substitutes were combined in these cases. Digital radiographs following surgical procedures were obtained using x-ray units equipped with flat panel DR or CR systems. Informed written consent was obtained from the parents of the children to use their digital radiographs for this study. A series of 47 consecutive children underwent synthetic ceramic bone graft substitute [HASi] implantation for various indications, of whom 30 children were included in this study. Those patients with copies of X ray films that were scanned, patients with plaster cast or fixation devices, and children who did not have standardized radiographs as per the protocol mentioned below were excluded due to the potential influence on pixel value measurements.

To validate the method of the radiographic imaging, standard exposure parameters including tube potentials (kV), tube time current (mAs), collimation, source to image distance (SID), and use of grid were considered. A standard aluminum step wedge and ceramic graft were placed in the collimated area, and exposures were manually and automatically selected by the machine using Automatic Exposure Control (AEC). Two Philips Digital Diagnost, (Philips Healthcare, Eindhoven, Netherlands) 1100 mA DR systems equipped with flat panel detectors were used to validate the study. These machines were periodically calibrated using Unfors Xi (Billdal, Sweden). For standardization, an aluminum step wedge and bare ceramic graft (HASi) was placed inside the collimated area adjacent to the imaged part of the patient (Figure 1). The validation also incorporated measurement of pixel values of the step wedge and graft material, obtained with different factors using the same machine, same exposure parameters using two different machines, and with the same factors and same machine. The step wedge was used as a quality control tool in order to see contrast differences on the image. An ROI (region of interest) on a single step and the graft would be enough to depict variations of x-ray intensity between two time points, or difference between outputs of two different machines. While analyzing the image, reproducibility of pixel values with the same or different size of ROI was studied.

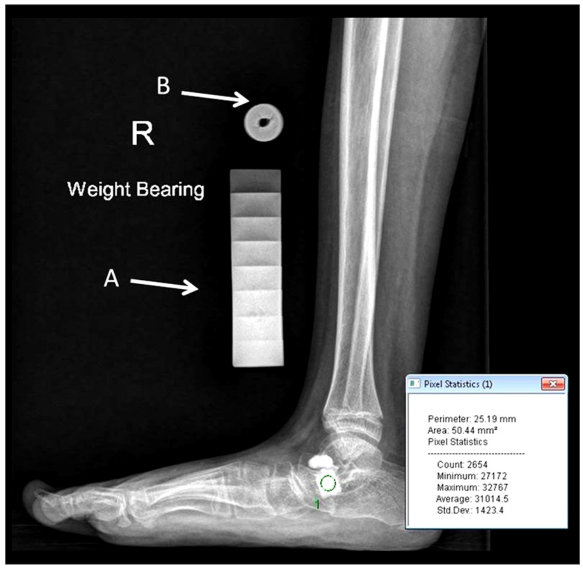


Figure 1. Radiograph standardized using a step wedge 'A' and bare graft (HASi) 'B' adjacent to the patient's leg in the collimated area.

The PVR was assessed for a given ROI using the technique described by Singh et al. [10]. Pixel values were obtained using the ROI tool available in the PACS system Centricity Enterprise Web (Version 3.0 (8.0.1400.37), GE medical systems, USA) (Figure 2). The PVR was calculated as the ratio of pixel value of graft (G1) to the average pixel value of proximal (P1) and distal native bone (P2) i.e., $G1 \div [(P1+P2)/2]$[10].

Assessment of graft Incorporation

To determine inter-observer and intra-observer reliability, PVR measurements were done by three independent observers – two pediatric orthopedic surgeons (A, B) and one radiologist (C). The measurements were done four weeks apart and a uniform protocol was followed by all observers based on guidelines established before reading each set of measurements. All observers followed similar criteria for selection of ROI size and region of measurement. PVR measurements were done for one set of radiographs at the point of plaster removal following surgery (8 weeks) and for the second set at the final follow-up visit. A PVR of ≤1 was assumed to indicate that the graft had achieved the native bone density and was completely incorporated (Figure 3).

Inter-observer and intra-observer variability for PVR measurement was assessed using the intra-class correlation coefficient. Healing of the graft was assessed radiologically by the van Hemert classification system [12], a five point grading system based on the stages of bone healing (Table 1). Spearman correlation coefficient was used to test the correlation between the PVR and van Hemert scores. Statistical analysis was done using SPSS (Statistical Package for Social Sciences), version 16.0.2.

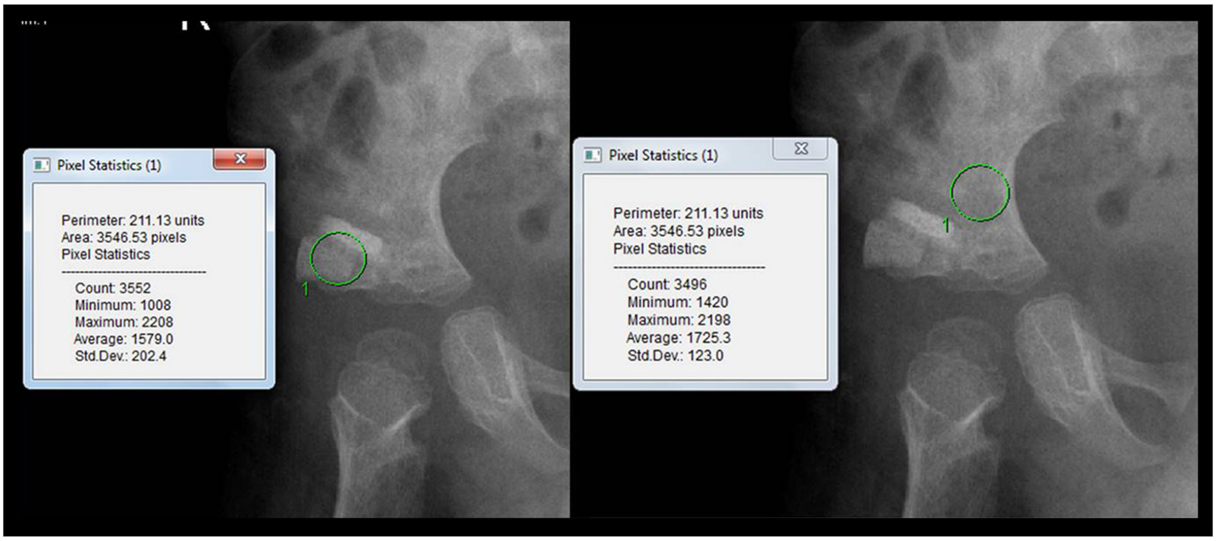


Figure 2. Pixel value measurement using ellipse ROI tool in PACS. The graft has a higher pixel value than the normal bone at 4-month follow-up.

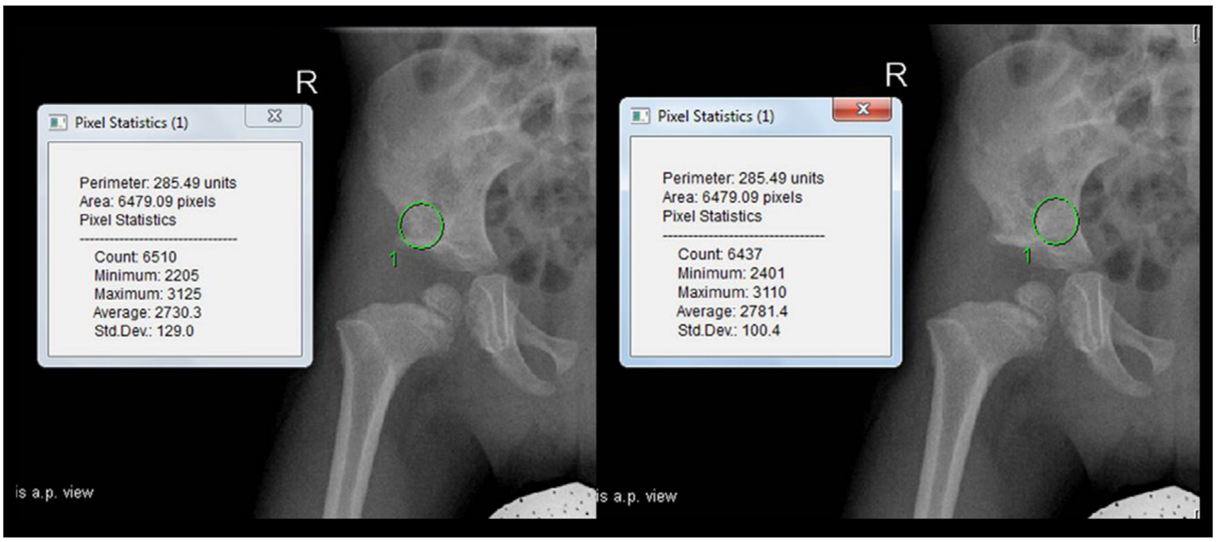


Figure 3. Follow-up pixel measurement at 12 months. The pixel values of the graft and normal bone have become equivalent.

Table 1. Stages of healing according to Van Hemert system.

Stage of healing	Name of the stage	Pathological status	Explanation
0	Immediate postoperative stage	Inflammation	Hematoma
1	Vascular phase	Soft callus	Osteopenic bone, rounded osteotomy sites, clear distinction between hasi and bone
2	Calcification stage	Soft and hard callus	Whitening of sites and blurred distinction between HASi and bone
3	Osteoblastic stage	Hard callus, remodelling	Distinction between bone slightly visible, healed osteotomy
4	Consolidation stage	Hard callus, remodelling	Full reformation, though osteotomy healed, HASi outline blurred
5	Full reformation	Remodelling	No sign of HASi

Adapted from Van Hemert et al. Knee, 2004; 11: 451–56 [12].

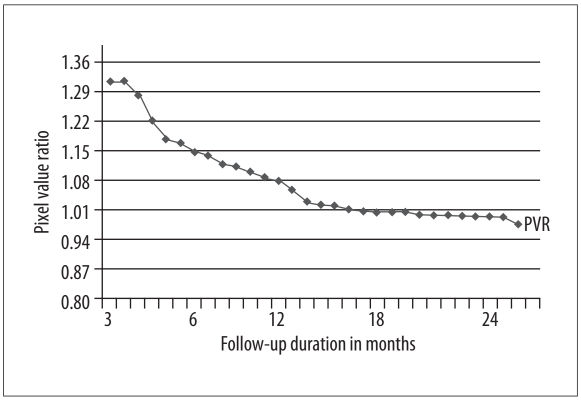


Figure 4. Serial pixel value measurements at follow-up show graft incorporation over time.

Results

The mean age was 8.09 years (range, 1 to 15 years). Follow-up duration ranged from 6 to 24 months. The mean PVR values (in the region of ceramic substitute) at the first follow-up and final visits were 1.471 and 1.079, respectively. Wilcoxon signed rank test for PVR data showed a statistically significant ($p<0.0001$) change in the density of the graft, when the PVR measurements at the first visit were compared with those at the last follow-up visit.

All subjects showed a pattern of gradual graft incorporation. Figure 4 indicates gradual incorporation of graft over a period of 2 years with decreasing density and PVR approaching 1. The intra-class correlation coefficient (ICC) for intra-observer reliability showed good agreement between the repeat measurements (A=0.86, B=0.92, C=0.91). The mean ICC for intra-observer reliability was 0.89. Inter-observer reliability between the three (A, B, C) observers was calculated for each pair of observers for

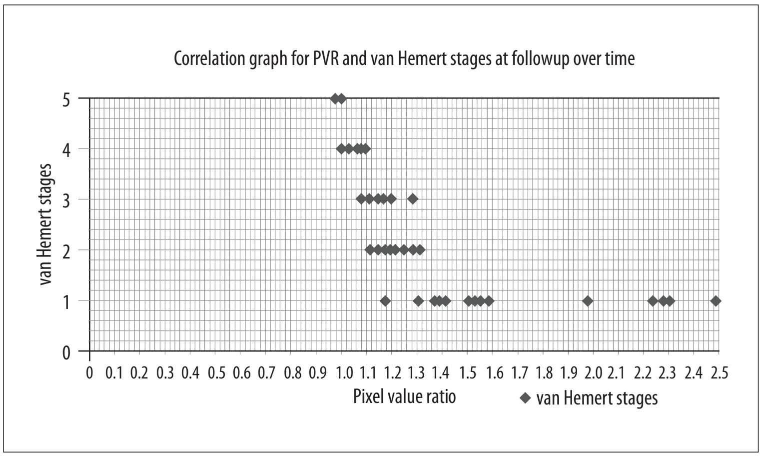
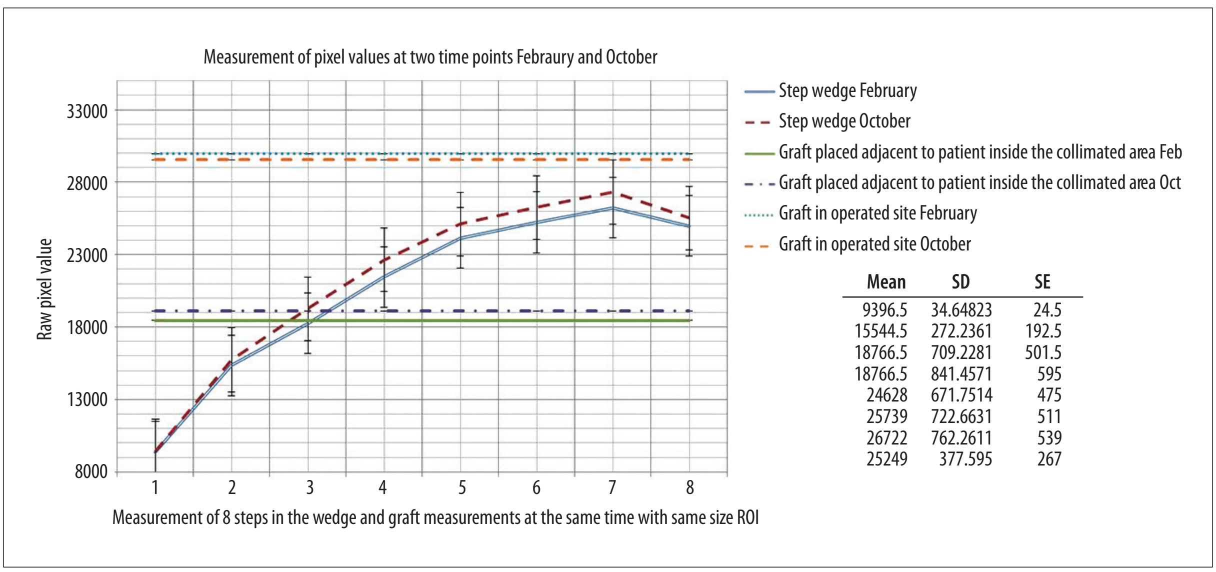


Figure 5. Negative correlation between van Hemert Scoring and pixel value ratio. R=0.9428 ($P<0.0001$).



Mean	SD	SE
9396.5	34.64823	24.5
15544.5	272.2361	192.5
18766.5	709.2281	501.5
18766.5	841.4571	595
24628	671.7514	475
25739	722.6631	511
26722	762.2611	539
25249	377.595	267

Figure 6. Illustrates the pixel values for 8 steps from the step wedge placed adjacent to the patient at two different time points. An average variation of 19% (12.8 to 31) in the pixel values was observed, if the same exposure factors and machine was used. The variation of the pixel values may be attributed to the back scatter radiation from the imaged area. A variation of 31% was observed in the pixel values of graft between 2 time points. Mean/Standard Deviation (SD) and Standard Error (SE) for step wedge given.

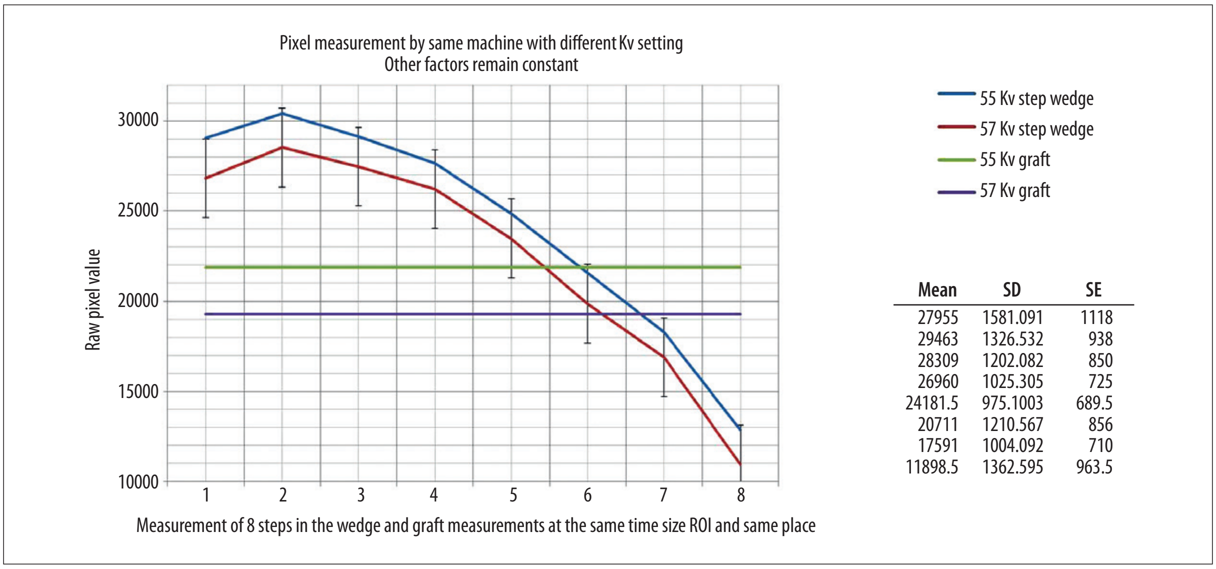


Figure 7. Illustrates the pixel value measurement for 8 steps from the step wedge using different exposure factors in the same machine using automatic exposure control. An average variation of 7.6% (5.2 to 14.9) in the pixel values was observed. A variation of 11% was observed in the pixel values of graft. Mean/Standard Deviation (SD) and Standard Error (SE) for step wedge given.

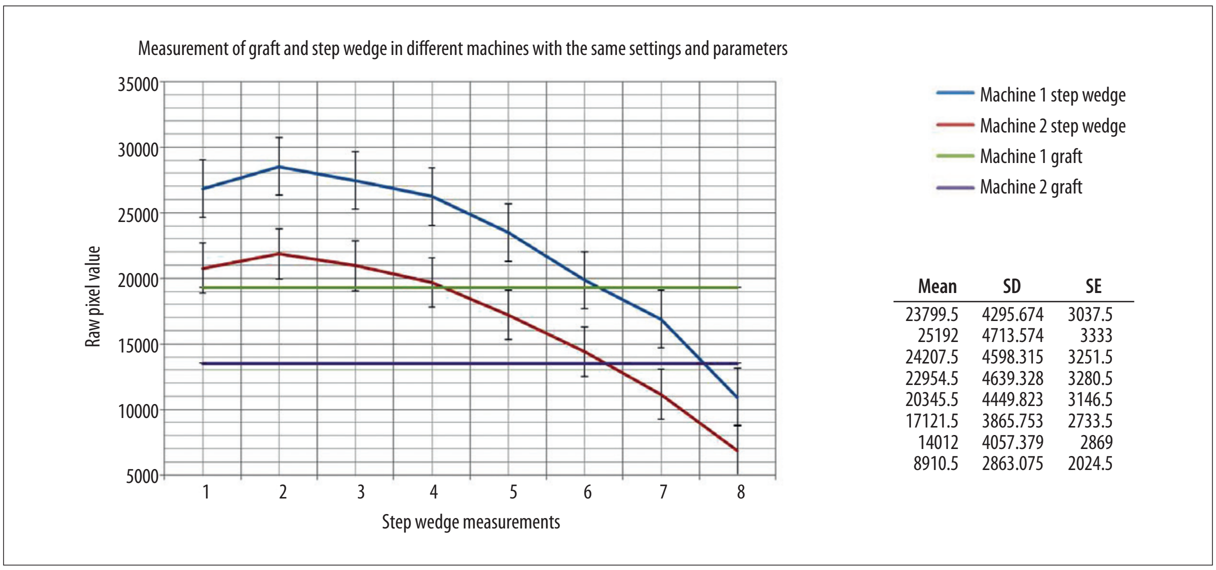


Figure 8. Illustrates the pixel value measurement for 8 steps from the step wedge using same exposure factors with different machines. An average variation of 27.5% (22.6 to 37) in the pixel values was observed. A variation of 30% was observed in the pixel values of graft. Mean/Standard Deviation (SD) and Standard Error (SE) for step wedge given.

both time points (A and B; B and C; C and A). Interclass correlation for inter-observer reliability for pairs of observers showed a strong agreement between the observers [first measurement – 0.69 (C *vs.* A), 0.78 (A *vs.* B), and 0.85 (B *vs.* C); second measurement – 0.74 (A *vs.* C), 0.82 (A *vs.* B), and 0.7 (B *vs.* C)]. Spearman correlation revealed an inverse relation between the PVR and the objective scores assessed using the van Hemert staging system (Figure 5). Spearman correlation coefficient was statistically significant ($r=-0.94$, $p<0.0001$) for van Hemert stages and PVR over the period of follow-up.

When validating the radiographic technique it was observed that the pixel values of the step wedge and graft were similar when the same machine, with a constant kV and mAs, was used, irrespective of the size of the ROI. Hence, the PVR (pixel value of step wedge/graft) was constant. However, pixel values changed with different parameters and settings in the same machine or in different machines with the same settings (Figures 6–8, respectively).

Discussion

The pixel value ratio was found to be a reliable indicator of bone graft incorporation in our study. It was reproducible and comparable with the radiographic scoring system of Van Hemert. However, it was influenced by individual machine parameters and thus requires a standardized protocol of acquiring radiographs and performing PVR measurements to avoid errors. We acknowledge that this can certainly be achieved in a research situation, but can be difficult in a busy clinical setup. It is also noteworthy that routine radiographs acquired for follow-up can be used for PVR measurement, and no additional imaging is necessary, if the imaging technique adheres to a standardized protocol.

Assessments of graft incorporation using radiographic classification systems are subjective and arbitrary, and are subject to inter-observer variability [13]. In our study, serial measurements showed that PVR correlated well with bone substitute incorporation. There was near perfect reliability and strong reproducibility in this measurement system. Initially, the PVR was greater than 1 for the radiopaque graft. However, this gradually decreased towards 1, as the radiopacity equaled that of the surrounding native bone. As the PVR decreased, the van Hemert scores for the stage of healing increased, indicating that PVR reduced with advanced healing.

The major limitation of our study was that measurement of PVR was derived from digital radiographs obtained using DR and CR systems. Our findings suggested a difference in PVR of the step wedge and graft material, when different machines or different parameters were used. In light of this finding, it is necessary to keep all parameters constant while using PVR as a tool for assessing graft incorporation. Another drawback of this method, which also exists for other techniques such as DXA scan and QCT assessment, is the interference caused by metallic fixation devices and the requirement of standardized patient positioning for all imaging protocols.

Another limitation of this study is the small sample size. We performed a pilot project, hence the small sample approved in the initial phase. Confounding factors such as soft tissue interphases, varying rates of healing in different regions of the body, the impact of the surgical technique, and the varying pathology were not taken into account in this study and thus limit the application of this technique at present to research settings, where parameters can be reproducibly controlled. The radiopacity of an image depends on the attenuation co-efficient of the material, which in turn influences the pixel values. In the case of bone, a decrease in density over a period of time due to immobilization can influence the pixel value ratio. A change in thickness of the overlying soft tissues over a period of time can also alter pixel values. However, these confounders are relevant for any serial radiological measurement technique.

The size and placement of ROI is crucial in PVR measurement. Some parts of a scaffold incorporate into the bone at a faster rate than others; hence, the ROI should be as large as possible in order to represent the entire scaffold, especially the margins that abut normal bone, as these lie in close proximity to the vascular interphase. With an irregularly shaped defect, such as a cavity, it would be ideal to have a tool that completely encircles the defect. Difficulty is also encountered when there is an implant placed across the scaffold. In such a case, taking a mean ROI on either side of the implant in two views (AP and lateral) would be ideal. The incorporation of a standard protocol to cover the maximum area of scaffold may avoid the necessity of having all radiographic views at the same time point. The main advantage of allowing measurements at different time points is that the radiologist can serially document graft incorporation at each follow-up visit.

We observed that random radiographs retrieved from PACS to measure the PVR may not be reliable due to lack of standardization. It is mandatory to acquire images with the same exposure factors during all visits, so that validity of the PVR measurements is not compromised. Without a standardized protocol, measurement errors will occur. The reader needs to call attention to the fact that despite variability of the pixel values (Figures 6–8), the van Hemert clinical stages and actual incorporation of graft corresponded well with the pixel values (Figure 5).

Further studies are required to improve this technique, before its application in clinical settings is possible. During all visits when radiographs are acquired, we suggest to use a standard step wedge or control object that should be placed in the collimated area adjacent to the patient. This will help ensure consistency in the measurement of pixel values from the given ROI of the bone, which could then be graded against a region of the step wedge. Following surgery, native bone undergoes reparative changes, but the step wedge kept adjacent to the patient stays constant irrespective of the local change in biology at the surgical site.

Conclusions

PVR was found to be a reliable method to monitor graft incorporation provided a machine with identical specifications and radiographic parameters is used for initial and follow-up radiographs. The placement of ROI should include the maximum surface of the graft, as the incorporation rates are different at different points. A step wedge or similar control object can ensure reproducibility of PVR measurements. Use of standard parameters can make PVR measurement a practical tool in research settings for interpretation of bone substitute incorporation without the need for additional advanced imaging.

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Conflict of interest

The authors declare that they have no conflict of interest.

References:

1. Brans B, Weijers R, Halders S et al: Assessment of bone graft incorporation by 18F-fluoride positron-emission tomography/ computed tomography in patients with persisting symptoms after posterior lumbar interbody fusion. EJNMMI Res, 2012; 2(1): 42
2. Lee JH, Jeon D-W, Lee S-J et al: Fusion rates and subsidence of morselized local bone grafted in titanium cages in posterior lumbar interbody fusion using quantitative three-dimensional computed tomography scans. Spine, 2010; 35(15): 1460–65
3. Pearcy M, Burrough S: Assessment of bony union after interbody fusion of the lumbar spine using a biplanar radiographic technique. J Bone Joint Surg Br, 1982; 64(2): 228–32
4. Seaman JA, Pluhar GE, Rose ND et al: Evaluation of scintigraphy to assess incorporation of intercalary cortical bone grafts in sheep. Am J Vet Res, 2009; 70(9): 1079–86
5. Leniz P, Ripalda P, Forriol F: The incorporation of different sorts of cancellous bone graft and the reaction of the host bone. Ahistomorphometric study in sheep. Int Orthop, 2004; 28(1): 2–6
6. Albert TJ, Lamb D, Piazza MR et al: MRI evaluation of fusion mass incorporation after anterior cervical bony fusions: Preliminary findings. Paraplegia, 1993; 31(10): 667–74
7. Andersen T, Christensen FB, Langdahl BL et al: Fusion mass bone quality after uninstrumented spinal fusion in older patients. Eur Spine J, 2010; 19(12): 2200–8
8. Wong RW, Rabie AB: A quantitative assessment of the healing of intramembranous and endochondralautogenous bone grafts. Eur J Orthod, 1999; 21(2): 119–26
9. Hazra S, Song H-R, Biswal S, Lee S-H et al: Quantitative assessment of mineralization in distraction osteogenesis. Skeletal Radiol, 2008; 37(9): 843–47
10. Singh S, Song HR, Venkatesh KP et al: Analysis of callus pattern of tibial lengthening in achondroplasia and a novel method of regeneration assessment using pixel values. Skeletal Radiol, 2010; 39(3): 261–66
11. Song S-H, Agashe M, Kim T-Y et al: Serial bone mineral density ratio measurement for fixator removal in tibia distraction osteogenesis and need of a supportive method using the pixel value ratio. J PediatrOrthop B, 2012; 21(2): 137–45
12. Van Hemert WLW, Willems K, Anderson PG et al: Tricalcium phosphate granules or rigid wedge preforms in open wedge high tibial osteotomy: A radiological study with a new evaluation system. Knee, 2004; 11(6): 451–56
13. Selby MD, Clark SR, Hall DJ, Freeman BJC: Radiologic assessment of spinal fusion. J Am AcadOrthop Surg, 2012; 20(11): 694–703