Periosteal Thickness around the Femoral Neck: a cadaveric study

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Abstract

Background: The femoral neck is a common osteoporotic fracture site. In this study we investigate changes in mean periosteal thickness with age, sex, side and circumference along the femoral neck. We hypothesised that: periosteal thickness declines with age, that the periosteum is thicker in males, is thickest inferiorly around the neck, and becomes thicker distally along the neck.

Methods and findings: Sixteen femoral necks from 9 cadavers (mean age 82.78, 5 female, 4 male) were sectioned, decalcified and stained. Periosteal thickness in 4 quadrants around the circumference, and along its length, was measured. The difference in mean periosteal coverage between sexes was found to be insignificant (p=0.2857), and did not appear to decline with age (p=0.7760). No significant changes in periosteal thickness were seen along the length of the neck (p=0.9594). There was, however, a significant difference in coverage around the circumference of the femoral neck (p<0.0001).

Conclusions: The differences in periosteal coverage around the circumference of the femoral neck reported here are open to several theories. There may be potential for pharmacological intervention, gene-related therapy or periosteal transplant to target specifically vulnerable sites.

Keywords: Periosteum; Femoral neck; Osteoporosis; Femoral neck fracture

Introduction

The periosteum plays a major role in bone growth and repair. Bone ossifies by two processes of ossification: membranous and endochondral. In endochondral ossification, the periosteum forms at the periphery of the bone by the perichondrium while in membranous ossification the periosteum forms directly from the mesenchyme [1,2].

Periosteum covers the entirety of the long bones, but is not present on sesamoid bones or intra-articular surfaces of the long bones [3]. In the femoral neck, periosteum is found to be present, and is continuous with the fibrous capsule of the hip joint [4]. The presence of periosteum along the femoral neck accounts for mineralising periosteal tissue, calcifying fibrocartilage and alkaline phosphatase expression along the femoral neck [5-7]. Periosteal circumference has been reported to increase with age, accounting for the growing thickness of the femoral neck in some subjects [8]. However, Shea et al. [9] reported calcification and capsular mineralisation between the femoral head and neck in the elderly, which may account for at least some of the increased femoral neck width seen with age, making it difficult to determine how much of the increase is due to mineralisation and how much is due to periosteal bone formation.

Bone strength and subsequent fracture resistance at the femoral neck is a result of expanding periosteum, which is controlled by osteogenic activity [9]. Power et al. [6] found that alkaline phosphatase was expressed at the femoral neck periosteal surface, which accounts for the mineralisation-related neck expansion seen with age. Their hypothesis that cases of hip fracture show a lower degree of femoral neck expansion was not proven, since it was found that periosteal bone formation at the femoral neck was a common sign in elderly women, even in the case of hip fractures.

Despite the accepted view that osteoporosis is a consequence of reduced osteogenesis [10], more recent research has found that increased bone resorption is the principle factor in bone fragility [11].

Periosteal resorption has been shown to occur significantly alongside bone growth [3]. Chondrogenesis and periosteal thickness have been shown to decrease significantly with age in rabbits [4]; it is reasonable to suppose that this process also occurs in humans, which would explain the anticipated decrease in periosteal thickness and bone strength seen in old age.

However, absolute bone size is a major determinant of fracture risk and increased bone size comes about by periosteal expansion leading to periosteal thinning. The proliferative nature of the periosteum could leave it open to pharmacological intervention with anabolic or anti-resorptive agents such as those used to increase bone density, and thus reduce the osteoporotic fracture risk.

The aim of this study is to elucidate the coverage of periosteum around and along the femoral neck, by investigating changes in mean periosteal thickness with age, sex, side and circumference along the femoral neck in cadaveric specimens; and whether this can be associated with relative osteoporotic fracture risk.

Materials and Methods

Sixteen femoral necks from 9 cadavers (4 males, 5 females, average age 82.8 years) were retrieved following anatomical examination (2 femoral necks were unusable due to hip replacement surgery) (Figure 1). The cadavers used in this study had been donated for anatomical examination and research under the Human Tissue Act (2004).

Specimen no.	Sex	Age at death (yrs.)	Recorded cause of death
1	F	53	Multi-organ failure, bronchopneumonia
2	F	68	Hodgkin's lymphoma
3	M	80	IHD, type II diabetes
4	M	85	Carcinomatosis, cholangiocarcinoma, IHD
5	M	86	Bladder cancer
6	M	88	Bronchopneumonia, heart failure
7	F	91	Old age
8	F	96	IHD, end stage heart failure
9	F	98	Breast cancer, old age

**Figure 1:** Details of the individuals sampled in this study.

The medical practitioners of all the donors were contacted for information on: past history of fracture, osteoporosis risk factors, drugs that increase the risk of osteoporosis, protective drugs against osteoporosis and relevant investigations, including bone fragility scans. There were no significant histories to report.

Sectioning & labelling

The femoral sections were removed from embalmed cadavers in the dissecting room. Following removal of subcutaneous fat and muscle to obtain a clean bone specimen, the femoral neck was removed by making two transverse cuts using an oscillating bone saw.

The greater trochanter of the femur was used as a reference point for making the distal cut, parallel with but just proximal to the base of the articular capsule, in line with the highest point of the greater trochanter. The proximal cut was made just below the expansion of the femoral head. The femoral neck specimens were cut into 1 mm sections using a Buehler IsoMet® low speed saw with a diamond wafering blade.

A red histological ink was used to make a dot on the anterior quadrant (on the superior face of the section). The remaining quadrants could then be defined as superior, posterior and inferior, respectively (Figure 2).
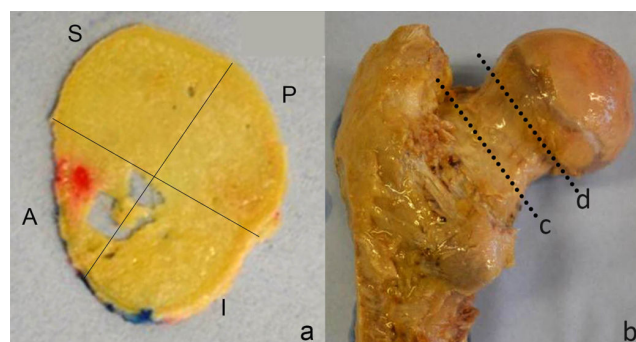

Figure 2: a): Section of femoral neck (a) showing anterior (A), superior (S), posterior (P) and inferior (I) borders. (b) Femoral neck showing orientation of sections and limits of femoral neck (c-d).

Decalcification, Staining & Microscopy

The sections were decalcified in 14% EDTA (ethylenediaminetetraacetic acid, VWR) in PBS (phosphate buffered saline), buffered to pH 7.2 with 0.1 M NH_4OH solution. Samples were left to soak in a 1-litre beaker containing the EDTA buffer on a hot plate set at 20°C, and agitated with a magnetic stirrer. The pH of the solution was monitored daily for 3 days and buffered when necessary. After 3 days the sections were removed and stained with Sudan black which highlights nerve fibres in the bone. This stain was found to be effective as the periosteum is dense in nociceptive nerve endings [12].

Stained sections were whole-mounted onto microscope slides with Histomount®, covered with a coverslip and placed in a laboratory oven at 70°C for 24 hours to dry.

Light microscopy and photography were carried at 10x magnification with a Zeiss Axioplan 2 upright microscope under mild fluorescence to enhance the differentiation between cortical bone and periosteum. Three approximately equally spaced measurements of periosteal thickness were taken from the middle section of each quadrant over a distance of approximately 200 μm (Figure 3). This gave a total of twelve readings from each section. The mean of the three readings from each quadrant was calculated, giving an average measurement of periosteal thickness for the anterior, superior, posterior and inferior quadrants, respectively.
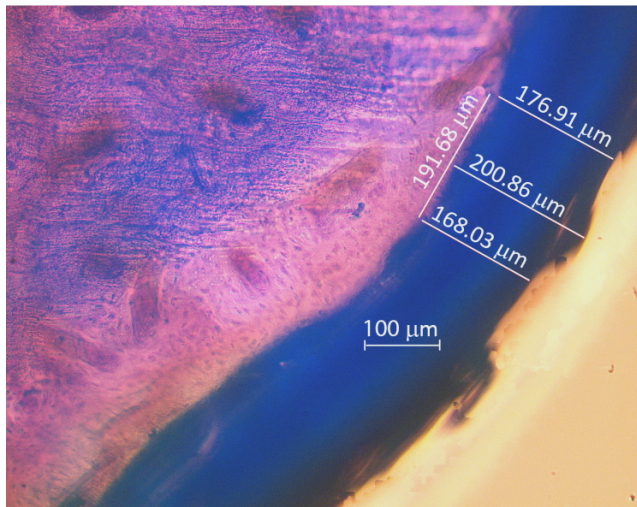
Figure 3: Segment of posterior quadrant stained with Sudan black. Three approximately equally-spaced measurements were taken at the mid-point of the quadrant.

Statistical analysis

All data were statistically analysed using GraphPad Prism 6 software. Mean periosteal thickness was compared to age, sex, side, anatomical border and distance along the femoral neck.

Results

No correlation was found between age and mean periosteal thickness (p=0.7760) (Figure 4).
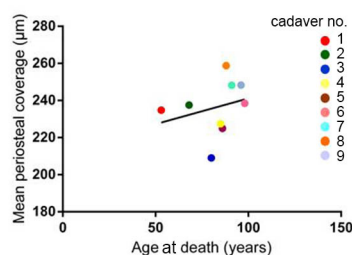
Figure 4: Linear regression of mean periosteal coverage (μm) against age (p>0.05).

Although the mean periosteal thickness was slightly greater in females, this finding was not significant (p=0.2875) (Figure 5). There was no significant variation in mean periosteal thickness between left and right femoral necks (p=0.5848).

**Figure 5:** Mean male and female periosteal coverage (μm) (± SEM, p>0.05).

In terms of mean periosteal thickness, a comparison between the four quadrants (anterior, superior, posterior and inferior) found that there was a highly significant difference in thickness, with the posterior quadrant having the thinnest covering, and the inferior the greatest (p<0.0001) (Figure 6). Nevertheless, a comparison between males and females of periosteal thickness at each of the four quadrants found there to be no significant variation between the sexes.
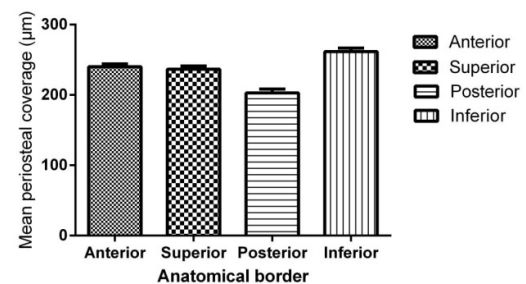
Figure 6: Mean periosteal coverage (μm) circumferentially, at anterior, superior, posterior and inferior borders of the femoral neck (± SEM, p<0.0001).

Finally, the coverage of periosteum was further analysed by comparing quadrant coverage on individual sections along the length of the femoral neck (Figure 7). The inferior border was found to be consistently the thickest region, and the posterior was the thinnest (p<0.0001). Variation between sequential segments along the length of the neck was found to be not significant (p=0.9594).
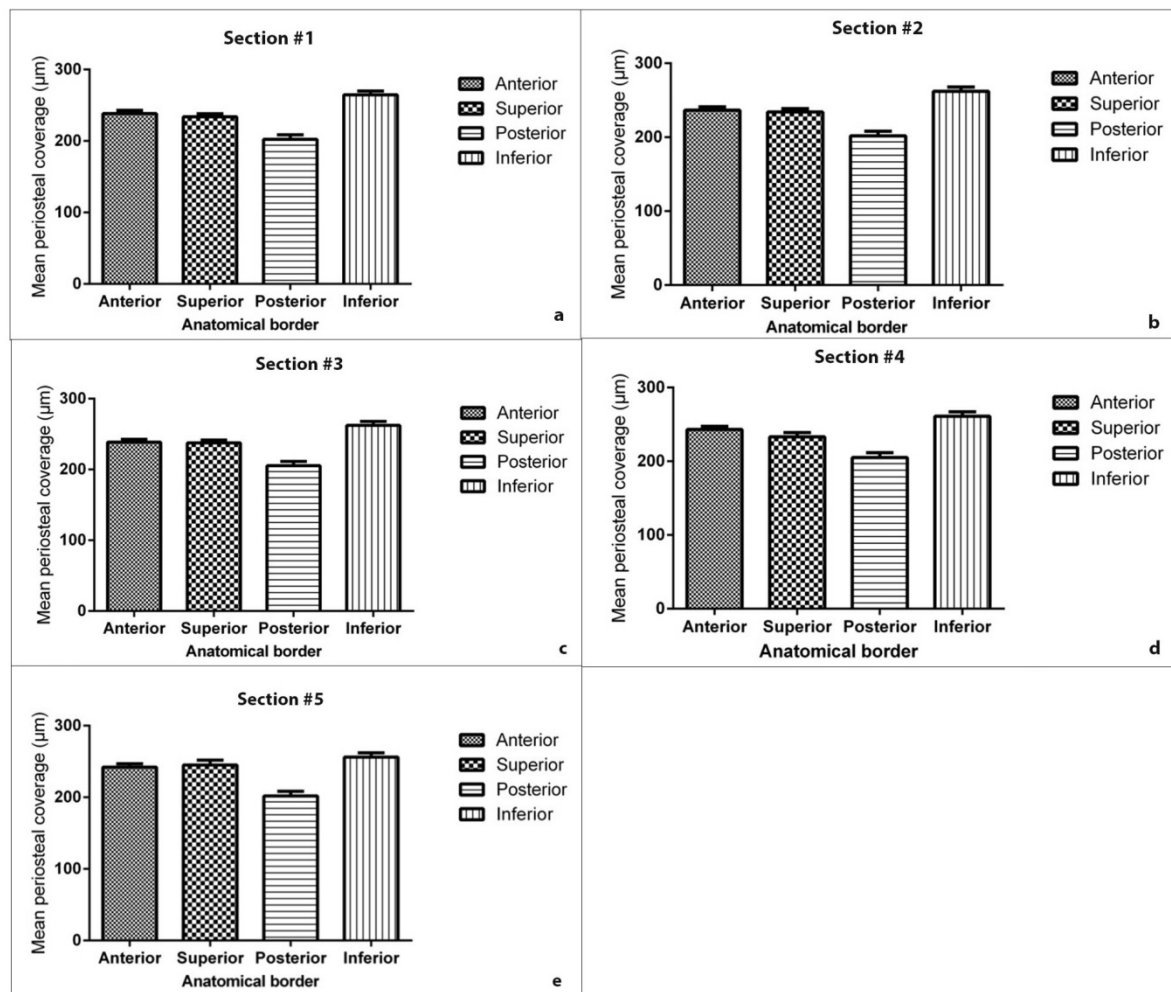
Figure 7: Mean periosteal coverage (μm) circumferentially at sections 1-5 (±SEM). Section 1 is the most distal.

Discussion

The risk of osteoporotic fractures increases with old age [13]. Therefore, it might be expected that coverage of periosteum at the femoral neck would decline with age. Furthermore, since sex is another osteoporotic risk factor, it might be expected that females would possess thinner periosteal coverage than males, due to the effects of menopause and subsequent oestrogen deficiency on osteoblast proliferation [14]. Contrary to expectations, the data reported here suggest that neither of these suppositions is in fact borne out by the experimental evidence.

Further analysis carried out to examine the periosteal coverage circumferentially and along the length of the femoral neck confirms our hypothesis that periosteal coverage varies circumferentially. We found that the periosteum is thickest at the inferior border which may be a consequence of load-bearing during gait.

During normal gait, the subcapital and mid-femoral neck regions of the proximal femur withstand the greatest biomechanical forces. Compressive stress increases propensity of fracture whereas tensile stress is less of a risk at the femoral neck [13]. A study by Lotz et al. [15] found that compressive stress upon the femoral neck is greatest inferiorly when walking, with low tensile force existing superiorly.

Periosteal apposition is also thought to occur with age, as a compensatory mechanism to increased mechanical stress [16,17]. Since the femoral neck experiences high compressive stress inferiorly through a lifetime of walking, quiescent periosteal tissue may be induced to resume bone apposition [18]. This mechanism confers strength to the femoral neck under stress and may account for the results obtained in this study.

Mean periosteal coverage was found to be thinnest at the posterior border, which may have been due to the disruption of the posterior periosteum on the femoral neck seen particularly in Salter-Harris type II fractures of the femur in children [18]. It could also be argued that due to minimal compressive stress posteriorly while walking [15], the periosteum in this region is not stimulated to grow, thus remaining relatively thin throughout life.

Mean periosteal coverage against age of death showed positive correlation, contrary to our initial expectations. Power et al. [6] also reported age-related periosteal bone formation at the femoral neck, as a result of elevated alkaline phosphatase expression; periosteal apposition can also increase due to increasing mechanical stress upon the femoral neck with age, as suggested by a number of studies [16-21].

Limitations

The sample size of 9 cadavers was too small to detect differences of periosteal coverings between the sexes. Indeed, the difference recorded was so minimal that a power calculation suggested that over 800 cadavers would be necessary to detect a significant difference – clearly way beyond the scope of this study. The data for variation round the circumference, however, was highly significant. Also, although the relevant medical history for all the donors was sought, it would have been useful to have access to a full history of past medications.

Conclusion

Age and sex appear to have no influence on the periosteal coverage of the femoral neck. However, there are significant differences in coverage round the circumference, with the posterior quadrant being the most sparsely covered, and the inferior quadrant having the thickest covering, consistent with the theory that these regions are subject to the least and greatest compressive forces during gait, respectively. This distribution is consistent along the length of the femoral neck.

There is much potential for research regarding the periosteum, with possible techniques to minimise the incidence of femoral neck fractures in the elderly. Pharmacological interventions, such as those used to increase bone density, could be targeted to maintain and increase periosteum thickness in osteoporotic bones. Furthermore, the manipulation of chondrogenic cells may in future preserve the proliferative potential of the periosteum with age. Krebsbach et al. [22] successfully induced fibroblasts with bone morphogenetic protein-7 (BMP-7) to produce bone *in vivo* through gene therapy-directed osteogenesis. Similarly, it has been observed that fibroblasts, osteoblasts and progenitor cells of the periosteum can be successfully grown *in vivo* and *in vitro* [23], and potentially transplanted into periosteum at sites where it is thinning.

Acknowledgements

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