CHAPTER 7

DKK-1 and Paget’s disease of bone

7.1 DKK-1 in serum of patients with Paget's disease of bone

Osteoblastic cells from patient's with PDB have recently been shown to secrete high levels of DKK-1 (Naot *et al.*, 2007). Therefore, serum samples from a group of patients with PDB were analysed for DKK-1 and compared to healthy age-matched controls. The PDB patients were in two groups, those that had received bisphosphonate treatment and those that had not (termed treated and untreated from here on). Mean age and sample size are shown in Table 7.1. Total serum ALP (tsALP) was also analysed as a measure of disease severity. Preliminary work optimised the DKK-1 assay for use with serum as assay results from plasma were significantly lower than the corresponding serum from the same sample. DKK-1 was also shown to be stable when serum samples were subjected to several freeze-thaw cycles.

TsALP values were found not to be normally distributed until log transformation was performed. Both treated and untreated males had significantly higher tsALP than control males ($P<0.001$, Fig 7.1A). Untreated males also had significantly higher tsALP than treated males ($P<0.05$). Untreated females had significantly higher tsALP than both control females ($P<0.001$) and treated females ($P<0.05$). There was no significant difference in tsALP between treated females and control females. There was no significant difference in tsALP between control males versus control females, untreated males versus untreated females, or treated males versus treated females. Combining male and female data for tsALP showed the same pattern of results (Fig 7.1B). Control subjects had significantly lower tsALP than both treated and untreated patients ($P<0.001$) and Untreated patients had significantly higher tsALP than treated patients $P<0.001$).

Table 7.1 Age and study sample size in PDB patients and controls. For age, values are shown as mean ± SD.

	N	Age
Control M	100	61 ± 9.8
Control F	40	74 ± 5
Untreated M	22	76 ± 12
Untreated F	10	79 ± 6
Treated M	14	80 ± 8
Treated F	12	76 ± 7

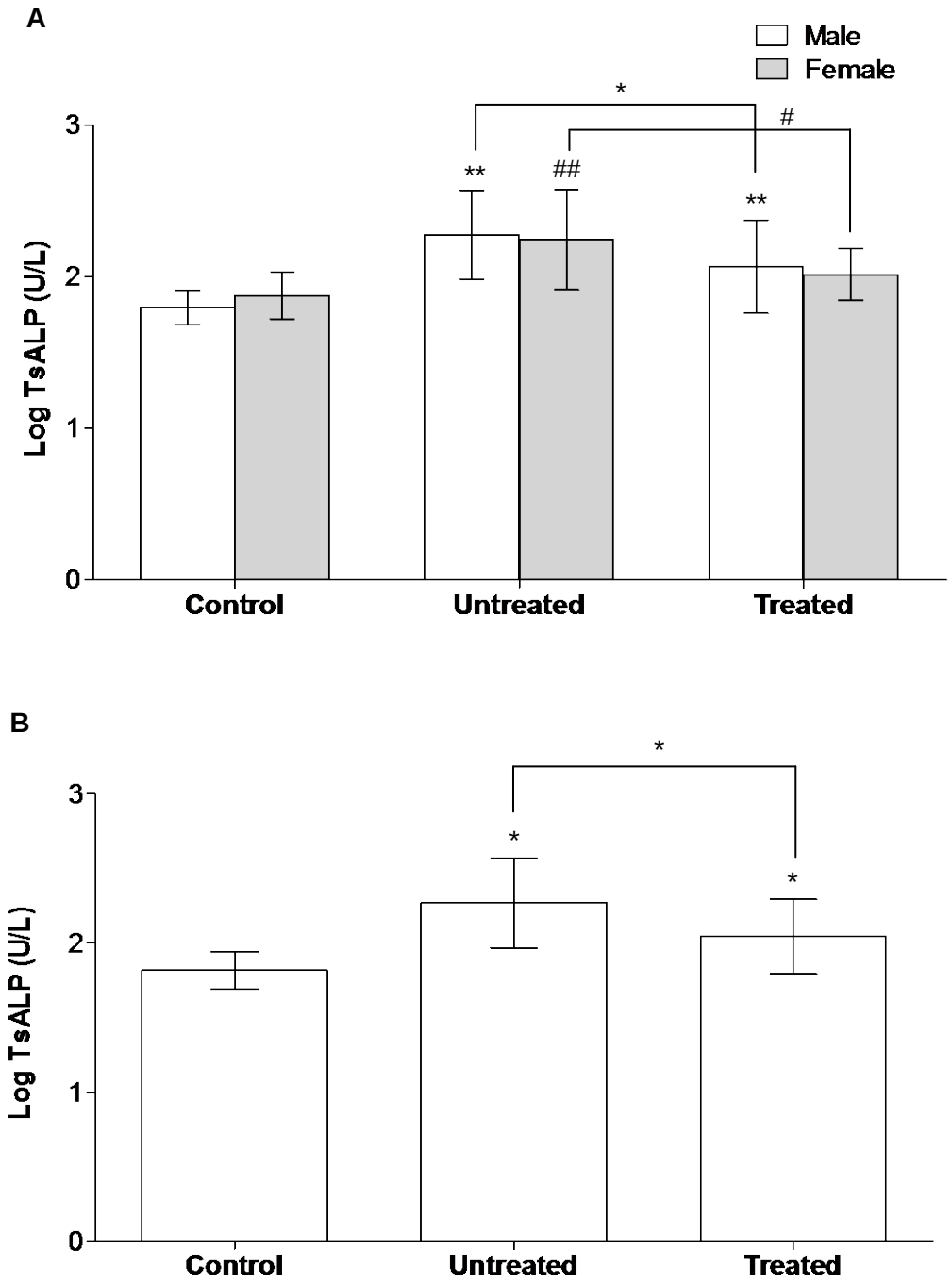

Fig 7.1 Log_{10} tsALP concentration in PDB patients and controls. A) tsALP concentrations showing differences between males (M) and females (F). *P<0.05 versus treated M and untreated M, **P<0.001 versus M control, #P<0.05 versus treated F and untreated F, ##P<0.001 versus control F (one way ANOVA using Tukey's multiple comparison test). B) Male and female data have been combined. *P<0.001 versus control (one way ANOVA using Tukey's multiple comparison test).

Serum DKK-1 values were found to be normally distributed. Both treated and untreated males had significantly higher serum DKK-1 than control males ($P<0.001$, Fig 7.2A). In females, treated and untreated patients also had significantly higher DKK-1 than control females ($P<0.001$). There was no significant difference in serum DKK-1 between untreated males versus treated males or untreated females versus treated females. Control males had significantly less DKK-1 than control females ($P<0.001$), but there was no significant difference in DKK-1 untreated males versus untreated females, or treated males versus treated females. Despite the significant difference in DKK-1 between control males and females, combining male and female data for DKK-1 also showed the same pattern of results (Fig 7.2B). Control subjects had significantly lower DKK-1 than both treated and untreated patients ($P<0.001$) whilst there was no significant difference between untreated and treated patients.

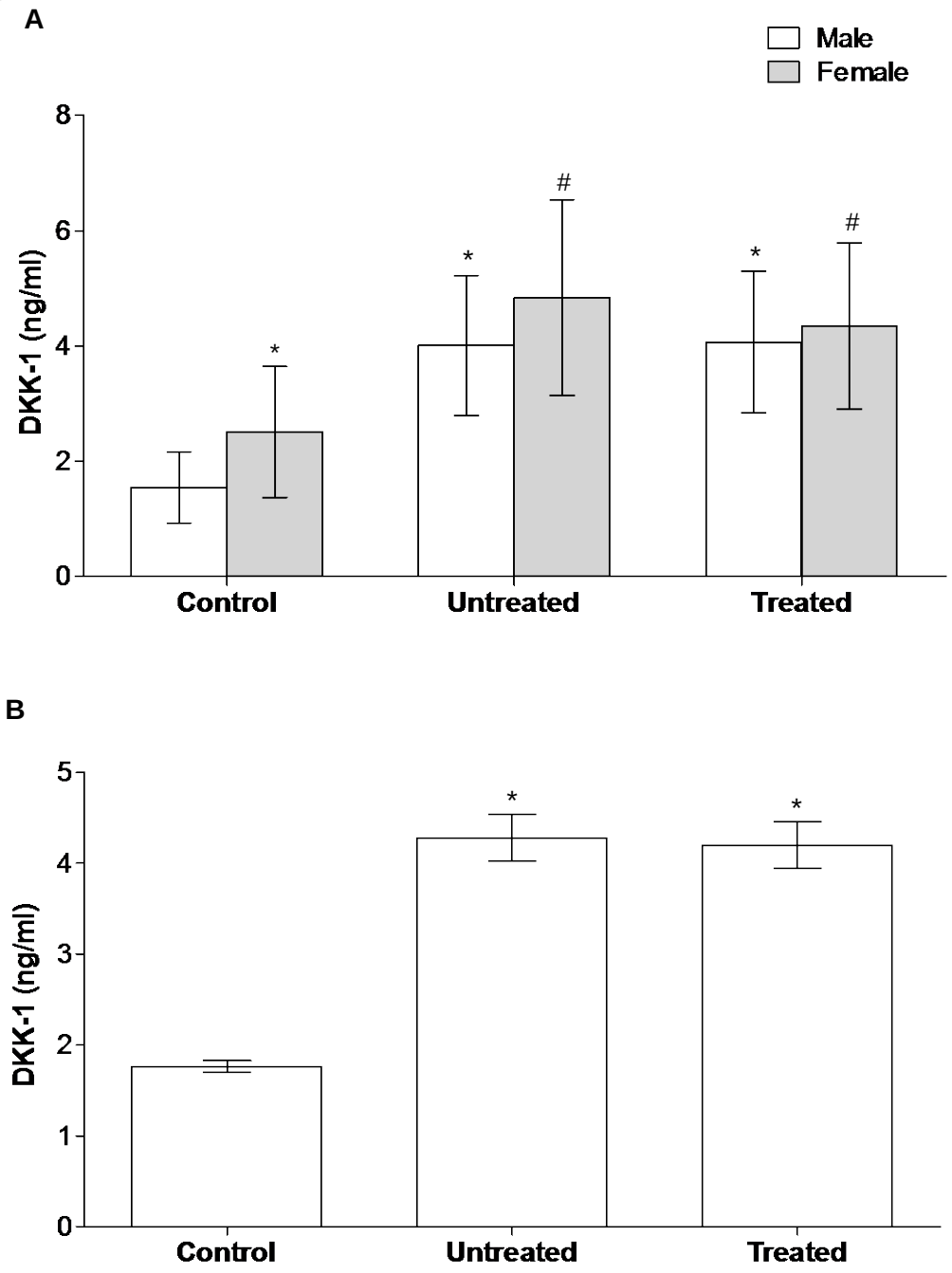

Fig 7.2 Serum DKK-1 concentration in PDB patients and controls. A) Serum DKK-1 concentrations showing differences between males (M) and females (F). *P<0.001 versus control M, #P<0.001 versus control F (one way ANOVA using Tukey's multiple comparison test). B) Male and female data have been combined. *P<0.001 versus control (one way ANOVA using Tukey's multiple comparison test).

7.2 Immunolocalisation of DKK-1 and β-catenin in patients with Paget's disease of bone

To further assess elevated levels of DKK-1 in patients with PDB, the immunolocalisation of DKK-1 was determined in formalin fixed paraffin embedded (FFPE) bone sections in both PDB and fracture patients. The immunolocalisation of β-catenin was also determined in the same sections to observe the effect of increased DKK-1 production on canonical Wnt signalling within the bone environment. Fracture callus shows a high rate of bone remodelling and so was deemed a suitable control for the increased remodelling rate observed in PDB.

Typical abundance of giant hyper-nucleated osteoclasts, erratic reversal lines, areas of paratrabecular fibrosis and disorganised osteoblast alignment were observed in PDB sections in contrast to fracture callus where these features were not seen (Fig 7.3). Positive staining for DKK-1 was found in the cytoplasm of plump active osteoblasts and newly embedded osteocytes in both fracture callus and PDB sections (Fig 7.4). Histologically, PDB sections appeared to have more spindle-shaped fibrotic cells within the marrow cavity than fracture callus (Fig 7.5). Remarkably a large amount of these fibrotic cells, particularly in areas of paratrabecular fibrosis also showed marked DKK-1 staining (Fig 7.5C). The presence of DKK-1 positive fibrotic cells increased towards the bone surface.

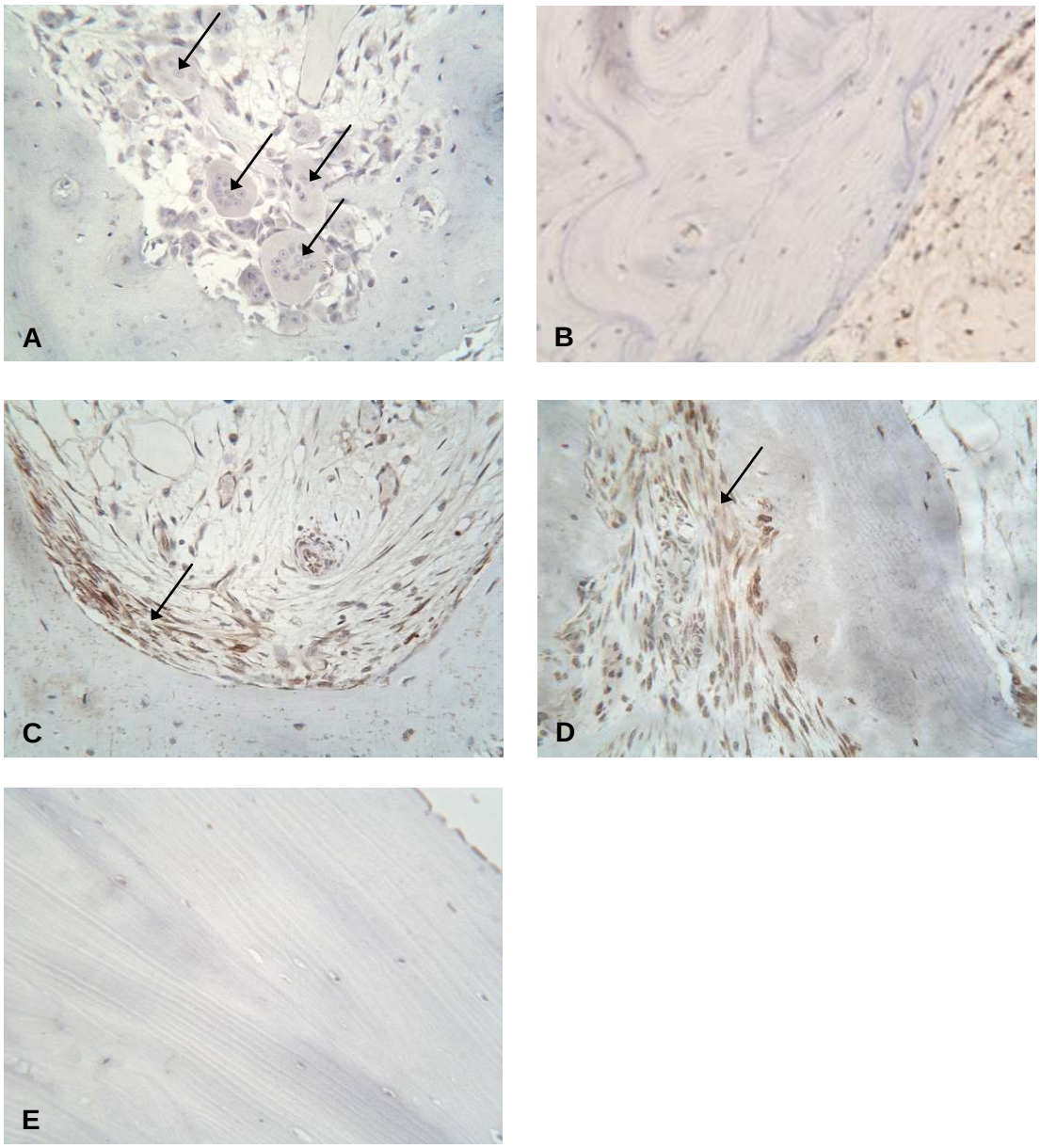

Fig 7.3 Key histological observations in FFPE bone sections from PDB patients. A) Giant hypernucleated osteoclasts (arrows indicate four osteoclasts, but many more can be seen). B) Erratic reversal lines caused by an increased rate of bone turnover. C and D) Paratrabecular fibrotic regions. Note how the osteoblasts appear to sweep over the bone surface (arrow) rather than sit neatly. D) Normal reversal lines from fracture callus section.

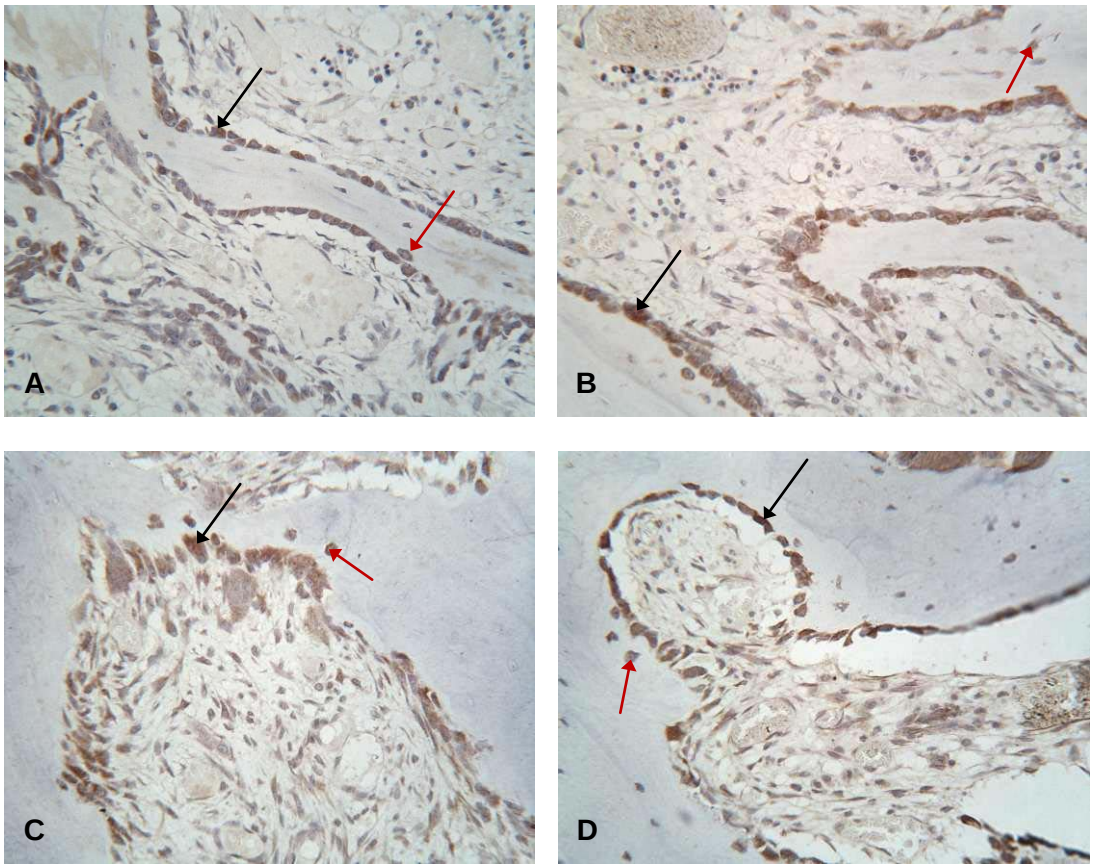

Fig 7.4 Immunolocalisation of DKK-1 in fracture callus and PDB. Positive staining of DKK-1 in plump active osteoblasts on the bone surface (black arrows) and newly embedded osteocytes (red arrows) in both fracture callus (A, B) and PDB (C, D). Note the neat arrangement of osteoblasts along the bone surface in fracture callus and the more disorganised nature of the osteoblasts in PDB.

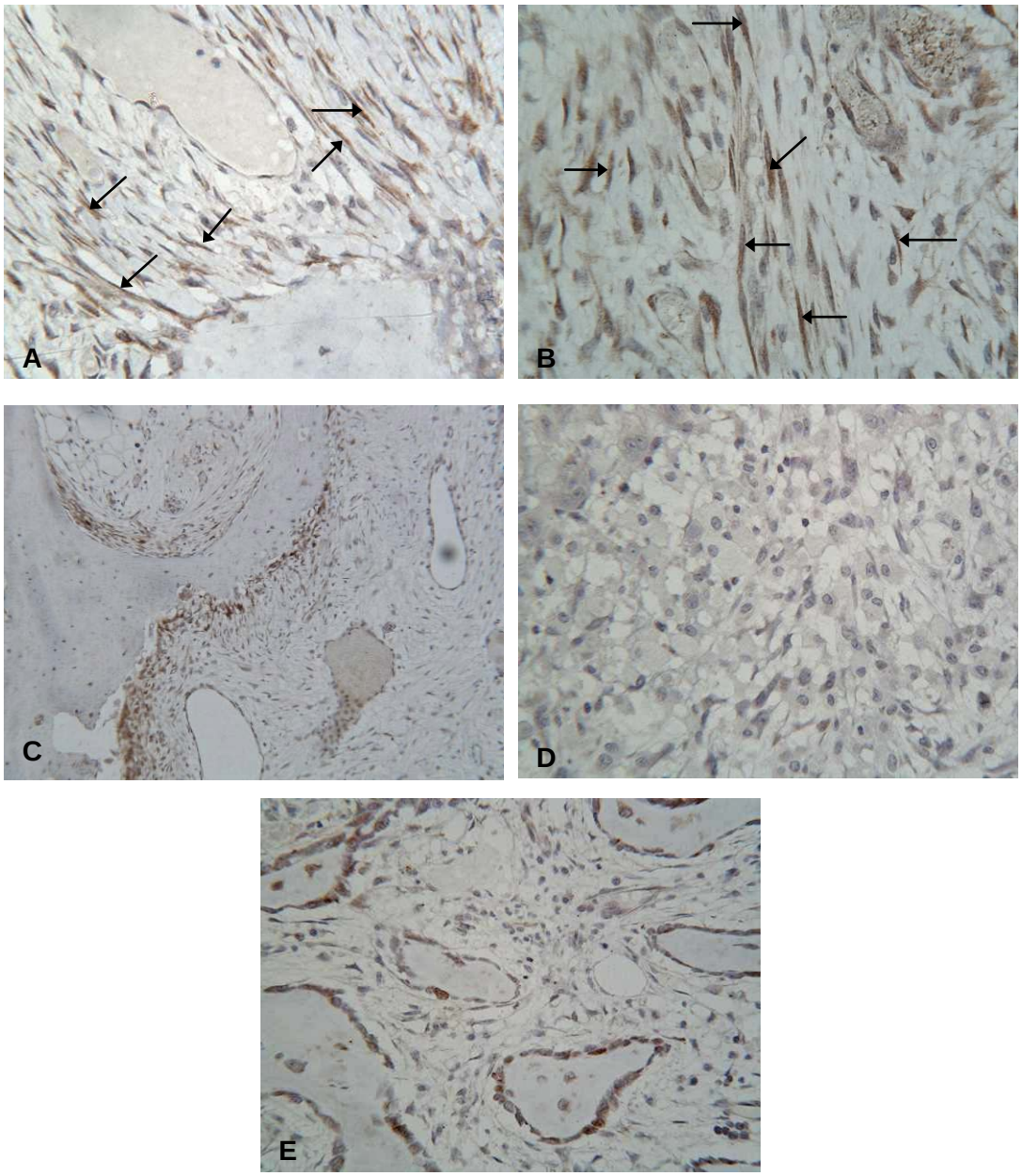

Fig 7.5 Immunlocalisation of DKK-1 in fibrotic regions of PDB. A and B) An increased amount of spindle-shaped fibrotic cells were observed in the bone marrow of PDB sections, of which a high proportion stained positive for DKK-1 (black arrows), particularly in paratrabecular fibrotic regions. C) DKK-1 staining in a paratrabecular region. Note how the intensity of positive DKK-1 cells increases towards the bone surface. D and E) Very few spindle-shaped fibrotic cells were observed in the bone marrow fracture callus, in which there was very little positive staining for DKK-1.

In fracture callus, positive staining for β-catenin was observed in plump surface osteoblasts on the intracellular membranes but not the membrane in contact with the bone (Fig 7.6). There was a slight positive stain for β-catenin in the cytoplasm of some plump surface osteoblasts, but not all (Fig 7.6). There was very little positive staining for β-catenin in fracture callus sections in any other cell types, including osteocytes and cells within the marrow area.

In PDB sections, positive staining for β-catenin was also observed on intracellular membranes of plump surface osteoblasts and not the surface in contact with the bone (Fig 7.7). PDB osteoblasts also showed much stronger cytoplasmic staining for β-catenin. Staining in PDB sections however was not restricted to the osteoblast; β-catenin was also detected osteocytes and in fibrotic cells within the marrow area (Fig 7.7). There was also evidence of slight positive staining for β-catenin in blood vessel endothelial cells in PDB sections (Fig 7.7).

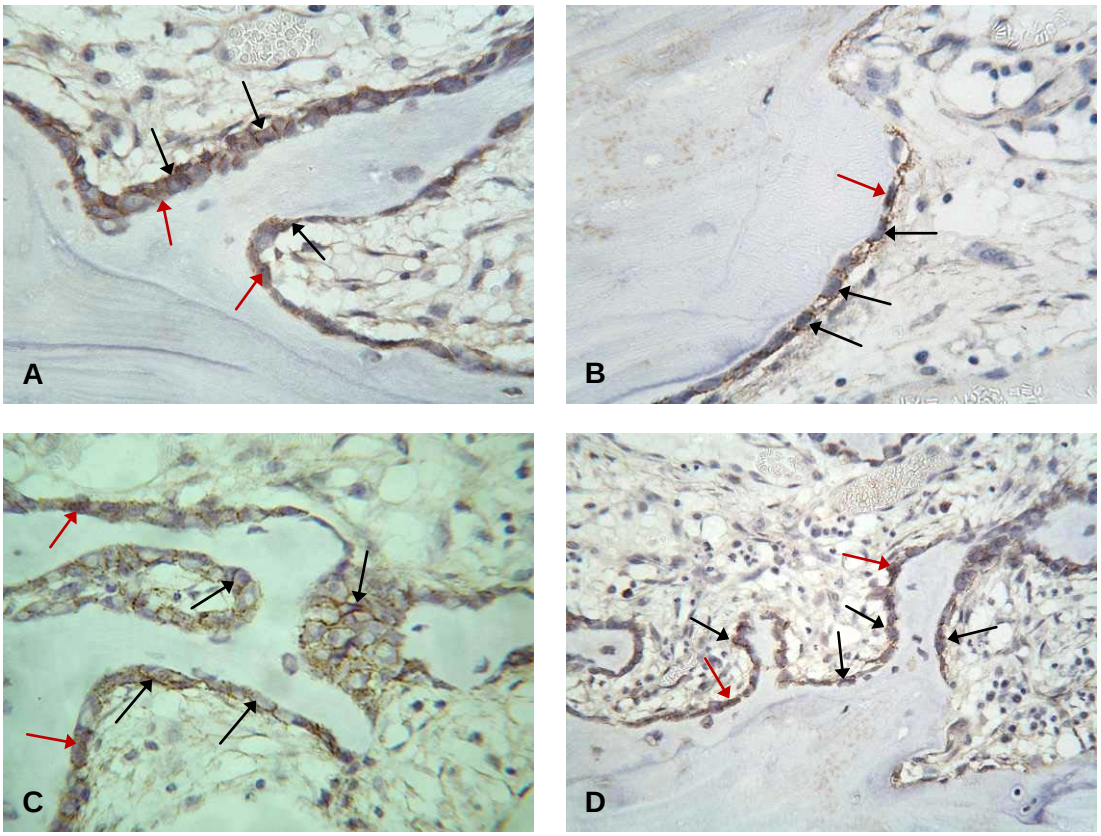

Fig 7.6 Immunolocalisation of β-catenin in fracture callus. A-D) Positive staining for β-catenin on the intracellular membranes of plump surface osteoblasts (black arrows), but not the membrane in contact with the bone surface. Some osteoblasts also show positive staining in the cytoplasm (red arrows). Osteocytes and bone marrow stromal area are negative for β-catenin staining.

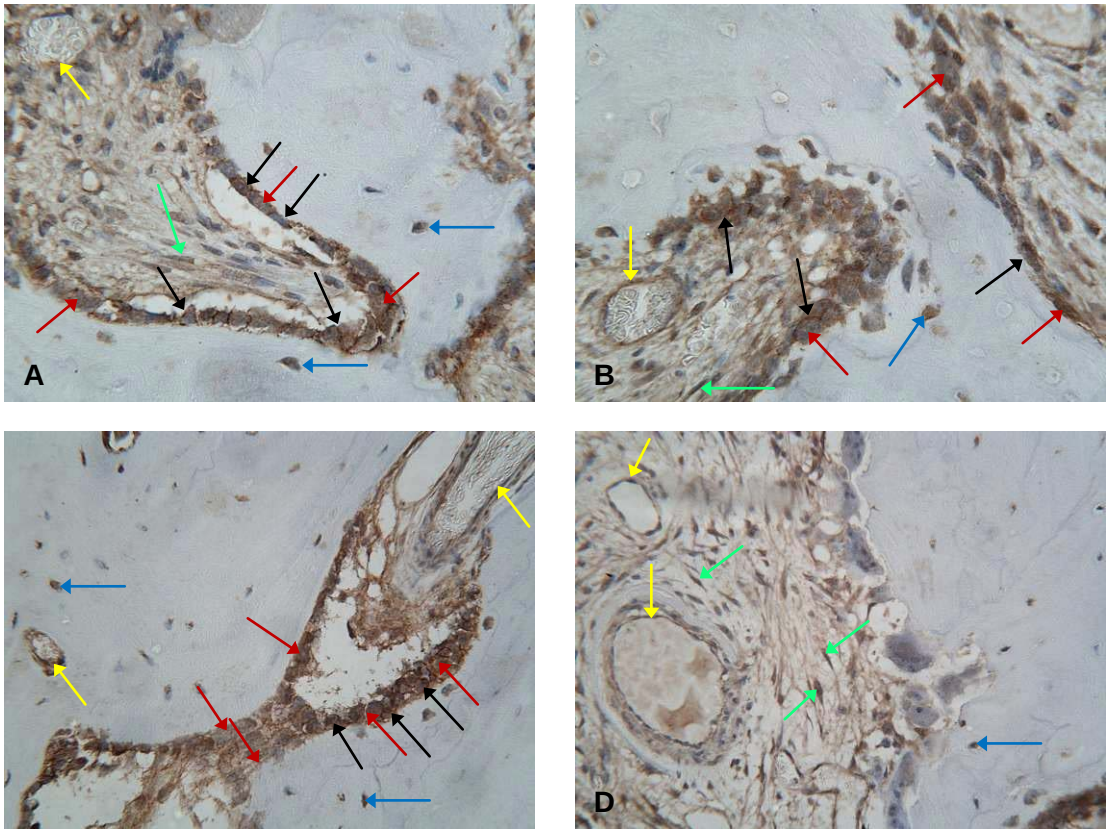

Fig 7.7 Immunolocalisation of β-catenin in PDB. A-D) Positive staining for β-catenin on the intracellular membranes of plump surface osteoblasts (black arrows), but not the membrane in contact with the bone surface. PDB osteoblasts also show positive β-catenin staining in osteoblast cytoplasm (red arrows), osteocytes (blue arrows), spindle cells within the bone marrow (green arrows) and blood vessel endothelial cells (yellow arrows).