RANKL EXPRESSION AS A PROGNOSTIC MARKER IN STAGE III GIANT CELL TUMOUR OF THE BONE

BY

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LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
BSP	Bone Sialoprotien
CALCR	Calcitonin receptor
Cbfa 1	Core binding factor alpa 1
COL	Collagen
CSF1R	Colony stimulating factor 1 receptor
CI	Confidence Interval
CT	Computed Tomography
DNA	Deoxyribonucleic acid
ER	Estrogen receptor
G0/G1/G2	Gap zero/Gap 1/Gap 2
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCT	Giant Cell Tumour
M-CSF	Macrophage colony-stimulating factor
MDM 2	Murine Double Minute 2
MIB-1	Mindbomb Homolog 1
MMP 1-13	Matrix Metalloprotienase 1-13
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
M phase	Mitosis phase
OC	Osteocalcin

OPG	Osteoprotegerin
OPN	Osteopontin
p53	Protien 53
PCNA	Proliferating Cell Nuclear Antigen
RANK	Receptor Activator of Nuclear Factor Kappa-B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
SD	Standard Deviation
S phase	Synthesis phase
T1	Time relaxation 1 second for MRI Image
T2	Time Relaxation 40 Micro Seconds for MRI Image
TGFBR2	Tumour Growth Factor-β type II receptor
TNF	Tumor Necrosis factor
TRAP	Tartrate-resistant acid phosphatase
VEGF	Vascular Endothelial growth factor

EKPRESI RANKL SEBAGAI PETANDA PROGNOSTIK DALAM PENYAKIT "GIANT CELL TUMOUR" TULANG TAHAP III

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Pengenalan: Penyakit "Giant cell Tumour" tulang adalah barah tulang yang bersifat agresif dan mempunyai kecenderungan untuk bertukar menjadi malignan. Ia mengandungi pelbagai jenis sel termasuk mononuclear sel stroma yang bersifat tidak stabil dan komponen reaktif iaitu monosit dan osteoklas. Di kalangan penduduk Asia termasuk Malaysia, ia dilaporkan lebih agresif dan mempunyai kadar yang lebih tinggi untuk merebak ke paru-paru. Sehingga kini, masih terdapat banyak keraguan dalam menjangka tahap kecenderungan untuk berulang dan perebakan ke paru-paru.

Objektif: Kajian ini bertujuan untuk menentukan keberkesanan ekspresi RANKL sebagai petanda prognostik dalam menjangka risiko untuk penyakit berulang dan perebakan ke paru-paru.

Bahan dan Kaedah: Kajian dan penilaian ekspresi RANKL secara "immunohistochemical" dijalankan ke atas 39 pesakit barah GCT tahap III. Terdapat 21 pesakit lelaki dan 18 pesakit perempuan dan purata umur adalah 36.2 tahun, di mana umur pesakit adalah dari 11 tahun hingga 66 tahun. Majoriti pesakit adalah berbangsa Melayu (85%) diikuti bangsa Cina (15%). Penyakit ini kebanyakannya terjadi di sekitar sendi lutut (46.2%) diikuti bahagian pergelangan tangan (17.9%). 35 daripada 39 (90%) pesakit telah menjalani pembedahan "wide resection" manakala 3 orang pesakit lagi menjalani reseksi intralesional dan hanya seorang pesakit telah menjalani amputasi tulang metakarpal.

Terdapat 10% daripada pesakit mengalami ulangan penyakit manakala 20% pula mengalami perebakan penyakit ke paru-paru. Sebanyak 5% mengalami kedua-dua masalah ulangan penyakit dan perebakan penyakit ke paru-paru. Antibodi poliklonal RANKL dari Santa Cruz Bioteknologi, telah digunakan untuk proses immunostaining. Pemarkahan dibuat berdasarkan sel yang positif bagi RANKL berlatar belakangkan 1000 sel stroma mononuklear. Semua data telah dianalsis menggunakan PASW versi 18.0.

Keputusan: Ekspresi RANKL dinilai berdasarkan peratus ekspresi (<25%, 25%-75%, >75%), kekuatan pewarnaan (1 hingga 3) dan jumlah markah bagi kedua-dua nilai (1-2= lemah, 3-4= sederhana, 5-6= kuat). Ekspresi RANKL didapati positif >75% pada 82% daripada jumlah sampel. Manakala, dari segi kekuatan pewarnaan, 48% mendapat markah 2 dan hanya 23% mendapat markah 3. 66.6% menunjukkan ekspresi RANKL yang kuat. Purata RANKL berdasarkan peratus pewarnaan, kekuatan pewarnaan dan jumlah kedua-dua nilai ialah 2.79 (SD 0.47), 1.95 (SD 0.72) dan 4.77 (SD 0.99). Nilai

purata ekspresi RANKL dari segi peratus pewarnaan adalah signifikan secara statistik dalam kumpulan kajian yang membandingkan penyakit berulang (p=0.009).

Kesimpulan: Ekspresi RANKL mungkin boleh digunakan sebagai parameter untuk menjangka risiko penyakit barah GCT tulang terhadap kecenderungan untuk berulang tetapi tidak untuk risko perebakan ke paru- paru.

Prof Dr Wan Faisham Nu'man Wan Ismail: Supervisor

Dr Md Salzihan Md. Salleh: Co-Supervisor

RANKL EXPRESSION AS A PROGNOSTIC MARKER IN STAGE III GIANT CELL TUMOUR OF THE BONE

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Introduction: Giant cell tumour of bone is an aggressive benign bone tumor composed of unstable spindle shaped neoplastic mononuclear stromal cells and reactive components osteoclast like giant cells. GCT in Asian population including Malaysia, presented with more aggressive tumour and higher rate of lung metastasis. Although numerous attempts have been made to predict the behavior of GCT, there are no definite biological or histological parameters to determine the prognosis or aggressiveness of this lesion.

Objectives: The aim of this study is to determine the presence of RANKL activity in aggressive type (stage III) GCT and its effectiveness as a prognostic marker in predicting risk of local recurrence and lung metastasis in GCT of bone.

Material and method: We evaluated 39 cases of stage III GCT of bone for RANKL expression using immunohistochemical staining. There were 21 males and 18 females patients and the mean age was 36.2 (ranged between 11 to 66 years old). Majority of the patients were Malays (85%) followed by Chinese (15%). 46.2% of the tumour occurred at a knee region followed by distal radius (17.9%). 35 out of 39 (90%) patients were treated with wide resection, 3 by intralesional resection and 1 cases of metacarpal GCT with extensive soft tissue involvement were treated by rays amputation. 10% of cases had local recurrence and 20% presented with or eventually developed lung metastasis. 5% of the cases presented with local recurrence and lung metastasis. Expression of RANKL was evaluated by immuno-histochemical staining techniques using the rabbit polyclonal antibody RANKL representing full-length RANKL human origin from Santa Cruz Biotechnology. The scoring was done by counting the positively stained cell in the background of 1000 mononuclear stromal cells. Data was analyzed using PASW version 18.0.

Result: RANKL expressions were evaluated by percentage of expression (<25%, 25%-75%, >75%), staining intensity (1 to 3) and final score of the two value (1-2= weak, 3-4= moderate, 5-6= strong). 82% of the sample expressed RANKL >75% from whole stromal cell population. Base on staining intensity, 48% scored 2, and only 23% had maximum intensity (3). 66.6% demonstrate strong RANKL expression. The mean value for RANKL according to percentage of staining, staining intensity and final score was 2.79 (SD 0.47), 1.95 (SD 0.72) and 4.77 (SD 0.99) respectively. The mean percentage

staining between no recurrence and with recurrence group was significantly different (p=0.009).

Conclusion: RANKL expression is generally high in stage III GCT and is a reliable prognostic markers in predicting the risk of local recurrence but not in lung metastasis.

Prof Dr Wan Faisham Nu'man Wan Ismail: Supervisor

Dr Md Salzihan Md. Salleh: Co-Supervisor

1.0 INTRODUCTION

Giant Cell Tumor of the bone (GCT) is regarded as a benign locally aggressive primary bone tumour that carries risk for local recurrences and pulmonary metastases (Osaka *et al*., 1997; Masui *et al*., 1998).

GCT of bone accounts for 4-8% of all bone tumours, however there is a usually high prevalence and aggressive presentations in Southern India and China, where it represents 20% of all primary bone tumour (Turcotte *et al*., 2006; Arnold *et al.*, 2011). In Malaysia, Faisham *et al*. (2006) and Ismail *et al.* (2010) have reported that most of cases presented were staged III GCT which were locally aggressive tumours and have high rate of metastasis to lung which range from 19.4% to 30%. The disease typically affects skeletally mature adults up to 40 years of age. Most of the tumours occur around the knee joint (50%) followed by distal radius and proximal humerus (Wullig *et al*., 2001).

The typical appearance of GCT is best demonstrated on conventional radiographs which show a lytic lesion located at epiphyseal region that has a well defined but non sclerotic margin, is eccentric in location, extends to the subchondral bone, and occurs in patients with closed physes (Murphey *et al.,* 2001; Turcotte *et al*., 2006; Arnold *et al*., 2011). There is no mineralized tumour matrix. MRI is crucial for determination of extraosseous extension, the marrow involvement and articular surface involvement and is important as part of local staging of the disease.

Based on radiological findings, Campannaci *et al*. (1987) have classified GCT into 3 stages. Stage III lesion are aggressive in nature. It shows a rapid progression of the

disease and is often associated with pathological fractures. The tumour has fussy borders, suggesting a rapid and possibly permeative growth. The tumour bulges into the soft tissue but the soft tissue mass does not follow the bone contour and is not limited by an apparent shell of reactive bone. On MRI, Stage III lesions are often characterized by cortical breakage and local soft tissue extension whereby in MRA (Magnetic Resonance Angiography) showed local increase in vascularity. Unfortunately, many authors including Campanacci *et al*. 1987 do not regard this staging system as predictive of the prognosis, because they found no correlation between radiography and local recurrence of GCT, or in other words its aggressiveness.

Macroscopically, GCT presents as a hemorrhagic, soft mass eroding bone. Microscopically, the tumor features a usually bland stromal cell population made of osteoblast lineages which are considered as a neoplastic factor in GCT and a second population of monocytes and eponymous multinucleated giant cells that are capable for bone resorption.

Numerous attempts have been made to predict the behavior of GCT of bone, however there has been no definitive clinical, biological or histological paramenter available that can give better prediction regarding the aggressiveness of the tumour in terms of potential for local recurrence and lung metastases (Faisham *et al.,* 2006).

Immunohistochemical studies give additional useful information in predicting the behaviour of GCT. There are various proliferation markers such as p53, Ki- 67, PCNA, MMP-2 and MMP-9 (Matrix Metalloproteinase), RANKL, VEGF and recently serum Tartrate-Resistant Acid Phosphatase 5b that had been studied with intention to correlate

with the aggressiveness of GCT.

RANKL is expressed by osteoblast lineage and become an important key effector molecule in promoting osteoclastogenesis, thus normal bone remodeling, whereby it binds with RANK on osteoclast surface membrane and trigger various intracellular signaling cascade and induce the differentiation of osteoclast and prolong their survivorship.

Various studies had been done using immunohistochemistry, immunofluorescence, RT-PCR assay and ex vivo stromal cells cultured confirmed that highly dense stromal cells of GCT expressed RANKL over the surface membrane of osteoblastic lineage. Wittrant *et al.* (2004) also found that RANKL expression is high compared to non-osteolytic bone tumours. However, there was no study, which evaluates and compares RANKL expression among GCT patient who presented with recurrence and lung metastasis.

We evaluated 39 archived tissue specimen diagnosed with GCT stage III, for positivity of RANKL in hope to find its correlation with local recurrences and lung metastasis, and probably justify the usage of denosumab among our local population.

2.0 LITERATURE REVIEW

2.1 Giant Cell Tumour (GCT) stage III

2.1.1 Definition

The World health Organization has classified GCT as "an aggressive, potentially malignant lesion", which means that its evolution based on its histological features is unpredictable (Szendroi, 2004). It is a heterogenous tumour composed of unstable spindle shaped neoplastic mononuclear stromal cells and reactive components that are monocytes and osteoclast like giant cells.

2.1.2 Epidemiology

GCT has a significant incidence, accounting for 20% of all benign bone tumors and 5% of all bone tumours, however there is an unusually high prevalence in southern India and China, where GCT represents 20% of all primary bone tumour (Turcotte *et al*., 2006; Arnold *et al*., 2011).

The natural history and behavior of GCT in Asian population was found to be more aggressive compared to Western population (Shih et al., 1998). In India, the incidence of GCT of bone was also reported to be high in the population which is about 20.3% of all primary bone tumours. In their series of 139 cases, they found as high as 51.1% were classified as Stage III GCT with 11.1% of local recurrence (Saikia *et al.,* 2011). The most

recent study in Japan also found more aggressive behavior of GCT where they reported the incidence of lung metastasis as high as 7.5% and malignant transformation as 2.7% (Takeuchi *et al.*, 2011). In Singapore, they had observed about 31% of local recurrence in their cases of GCT of bone (Lim and Tan, 2005). In Malaysia, Faisham *et al*. (2006) has observed that most of the cases were stage 3 GCT of bone which were locally aggressive tumours and more likely to metastasize to lung. In his series, about 30% of all the cases had pulmonary metastases. Ismail *et al.* (2010) has also found as high as 19.4% of pulmonary metastasis in his study of 39 cases.

The tumor appears in skeletally mature individuals with its peak incidence in the third and fourth decade of life (Carrasco and Murray *et al.*,1989; Salzer-Kuntschik et al., 1998). 80% of cases occurring between 20 and 50 years of age. Only 13% of cases occur in patients over the age of 50 years and less than 3% of cases occur before the age of 14 years (Turcotte *et al*., 2006). GCT in patients with an open growth plate is very rare and accounts for 1.5% of all GCTs (Picci *et al*., 1983). Although some studies show a slight female predominance, most support that there is no sex predilection in GCT (Turcotte *et al.,* 2006; M. Wulling et al., 2001).

The main localization of GCT is the epiphysis of long tubular bones (Mirra *et al.,* 1989; Delling *et al.,* 1997). However, in children with open physes, GCT of bone may be centered in the metaphysis and may abut the physis.. Nearly 50% of cases occur in the region of the knee including distal femur and proximal tibia. 15% of cases have been reported in flat bones such as the pelvis, sacrum, spine, ribs, and skull. Less than 1% of cases have been reported in the scapula. GCT may occur in the skull or pelvis secondary to Paget disease (Hoch *et al.,* 2007). The bones of the hands and feet are uncommon

locations, with a prevalence of less than 2% (Biscaglia *et al.*, 2000). GCT of the spine and sacrum is rare and is reported in less than 3% of cases (Kwon *et al.*, 2007). Multicentric GCT has been reported in less than 1% of cases, with lesions often located in the distal extremities, particularly the hands and feet.

Statistically, 80% of GCTs have a benign course, with a local rate of recurrence of 20% to 50%. About 10% undergo malignant transformation at recurrence and 1% to 4% gives pulmonary metastases even in cases of benign histology (Szendroi, 2004). In rare instances about 1-3% can transform into the malignant sarcoma phenotype with equal disease outcome (Werner, 2006; Dickson *et al.*, 2008).

2.1.3 Pathophysiology

GCT is a heterogeneous tumours that are composed of several cell types. A defining feature of the lesion is the presence of numerous multinucleated giant cells that are uniformly distributed amongst mononuclear spindle-like stromal cells and other monocytes. The giant cells are principally responsible for the extensive bone resorption by the tumour. However, the spindle-like stromal cells chiefly direct the pathology of the tumor by recruiting monocytes and promoting their fusion into giant cells. The stromal cells also enhance the resorptive ability of the giant cells (Robert *et al.*, 2013).

The spindle-like stromal cells are actually the neoplastic component of the tumor, owing to their ability to readily proliferate in culture (Goldring *et al.*, 1987; Wulling *et al.*, 2003) and their capacity to form tumors in mice. Collectively, the stromal cells show positive expression of bone sialoprotein, osteonectin, osteopontin, osterix, and the runt-

related transcription factor 2 (Runx2) but only occasional expression of osteocalcin. The stromal cells are themselves a heterogenous population and may consist of cells at multiple stage of differentiation including osteoblast.

The synthesis of giant cells from hematopoietic precursors is directed by spindle-like stromal cells in a process that closely mirrors osteoclastogenesis. Namely, the stromal cells produce chemokines, including stromal cell-derived factor-1 (SDF-1) (Liao *et al.*, 2005) and monocyte chemoattractant protein-1 (MCP-1) (Zheng *et al.*, 1998) that recruit monocytes to the tumor site. Following recruitment of monocytes, the stromal cells promote their proliferation and differentiation through the synthesis of macrophage colony-stimulating factor (M-CSF) (Atkins *et al*., 2000, Miyamoto *et al*., 2000) and potentially the recently- discovered interleukin (IL)-34 (Baud'huin *et al.*, 2010), which act via the colony stimulating factor 1 receptor (CSF1R) expressed on the monocytes. M-CSF also induces RANK expression on monocytes (Arai *et al.*, 1999). Cellular fusion of the monocytes is initiated by RANK ligand (RANKL), which is a membrane-bound member of the tumor necrosis factor (TNF) superfamily and is expressed by the spindle-like stromal cells (Atkins *et al*., 2000; Roux *et al.*, 2002, Miyamoto *et al*., 2000,; Huang *et al.*, 2000). Its expression is characteristically increased in GCT compared to controls (Morgan *et al*., 2005; Takayanagi *et al.*, 2000), and giant cell formation begins following it's binding to RANK.

Besides that, Osteoprotegerin (OPG), a soluble decoy receptor for RANKL, is also expressed by the stromal cells (Atkins *et al*., 2001) however, its presence does not entirely compensate for the increased RANKL expression.

Indeed, the giant cells express tartrate-resistant acid phosphatase (TRAP) (Anazawa *et al.*, 2006; Mii *et al.*, 1991), cathepsin K (Drake *et al*., 1996), and carbonic anhydrase II (Zheng et al., 1993, Kotake et al., 1996), as well as many receptors that are characteristic of osteoclasts, including the receptor activator of nuclear factor-κB (RANK) (Atkins *et al.*, 2006; Roux *et al*., 2002), the calcitonin receptor (Collier *et al*., 1998; Nicholson *et al.*, 1987) and the αvβ3 integrin. Moreover, the giant cells are capable of bone resorption (Balke *et al*., 2010) and may occasionally show numerous infoldings under electron microscopy that resemble the ruffled membrane of true osteoclasts (Kanehisa *et al*., 1991; Steiner *et al*., 1972). Perhaps the most significant difference between these cells and other osteoclasts is that the giant cells can be considerably larger, containing hundreds of nuclei (Zheng *et al.*, 2001, Gupta *et al.*, 2008).

The giant cells express the requisite bone-resorbing components of osteoclasts: cathepsin K (Drake *et al.*, 1996; Lindeman *et al.*, 2004) which largely degrades the organic components of bone (Garnero *et al*., 1998), vacuolar H^+-ATPase (V-ATPase) (Lindeman *et al*., 2004; Kotake *et al*., 1996; Morgan *et al.*, 2005), which demineralizes the crystals of hydroxyapatite (Blair *et al.*, 1989) and TRAP (Mii *et al.*, 1991), which may act by dephosphorylating bone matrix proteins and assisting in osteoclast migration (Janckila *et al.*, 2009). The presence of these enzymes suggests that giant cells resorb bone in a manner that is consistent with traditional osteoclasts.

2.1.3.1 Growth factor in stromal cells GCT

As mentioned, the spindles like stromal cells are believed the neoplastic element of GCT. Evidence has been offered that the stromal cells in GCT are the only persisting cell type in cell culture (Zheng *et al*., 2001; Robinson *et al*., 1996) and able to degrade bone matrix either independently or synergistically with osteoclast like giant cells. A recent report described cytogenetic aberrations including telomeric fusion, aneuploidy, and chromosome deletion in GCT stromal cells (Zheng *et al*., 1999) which further suggesting that telomere instability may participate in the development of GCTs.

Given these numerous genetic alterations, the status of the p53 tumor suppressor and other cell cycle regulators may also contribute to the progression of GCTs. Wu et al. reported that p53 is commonly mutated in GCTs. In contrast, other analyses have suggested that p53 is typically not mutated in primary lesions (de Souza *et al*., 1999, Osaka *et al*., 2004) although there is evidence indicating mutations in stromal cell p53 may be correlated with local recurrence, malignant transformation, and metastasis of the tumor (Masui *et al.*, 1998, Gong *et al.*, 2012).

Besides genetic abnormality, stromal cells also produce various growth factor for promoting recruitment of monocytes thus formation of giant cells which later contribute to resorptive capacity of osteoclast like giant cells and determine the aggressiveness of the tumour. Examples of the chemokines produce are stromal cell-derived factor-1 (SDF-1) (Liao *et al*., 2005) and monocyte chemoattractant protein-1 (MCP-1) (Zheng *et al*., 1998) that recruit monocytes to the tumor site and aforementioned RANKL.

Study done by Knowles et al; 2008, showed that higher expression of VEGF by stromal cells induce by local hypoxia, which induces angiogenesis from existing vasculature and resulrs in the formation of numerous small blood cells that are lined by CD31 and CD34-positive endothelial cells (Zheng *et al*., 2000). This VEGF could initiate a positive feedback loop of (pre)-osteoclast recruitment and act in an autocrine manner to enhance the pro-osteoclastogenic phenotype.

Additionally, numerous MMPs have been identified within GCTs, including MMP-2 and MMP-9, MMP-13, MMP-1, MMP-3, MMP-4, and multiple cathepsins. Despite the multitude of MMPs expressed within GCT, there is little actual evidence pertaining to any specific roles within the tumor. It is known that MMP-2 expression is correlated with the vascular fraction of the tumor (Strauss *et al.*, 2004), and MMP-9 may participate in vascular invasion by the giant cells (Ueda *et al*., 1996). However, given the established functions for cathepsins and MMPs in tumor angiogenesis, normal bone resorption, and osteoclastogenesis, it is likely that they perform similar tasks in GCT and actively participate in the resorption process by giant cells and may play a central role in catabolism of extracellular matrix macromolecules. Study by Ghert et al; 2007 suggest that the stromal cells produce the matrix degrading proteases MMP-2 and MMP-9, and these proteolytic factors are active in vitro and concluded that the stromal cells may therefore play a central role in degradative processes responsible for osteolysis in GCT of bone.

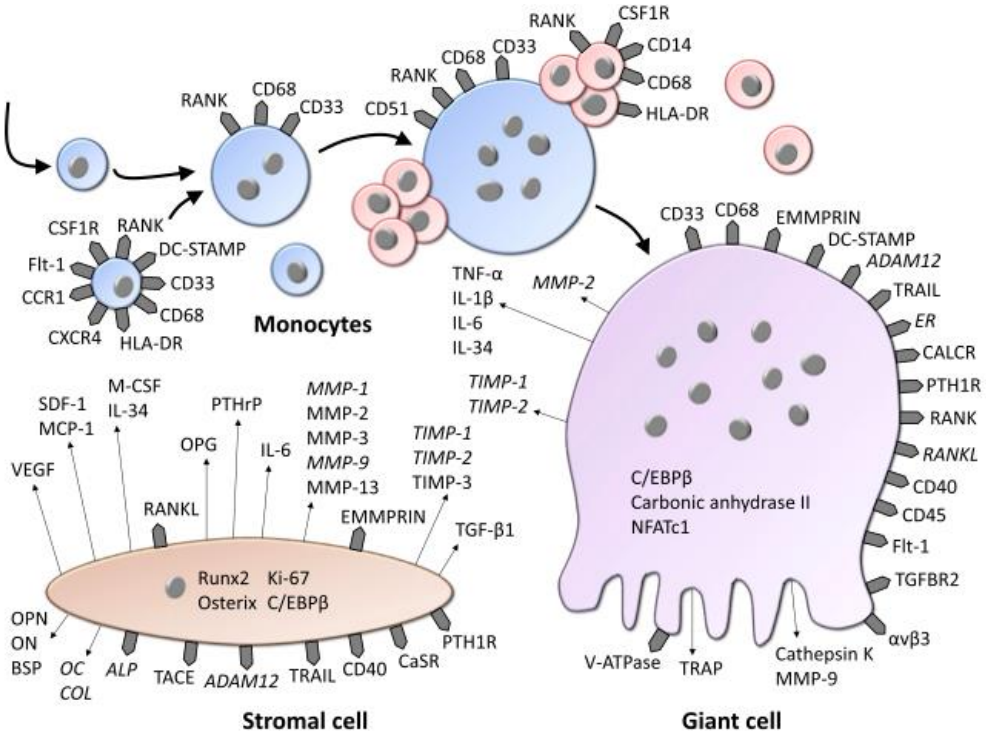

Plate 1: Schematic representation of GCT indicating expression of multiple key components by the spindle-like stromal cells, the monocytes, and the multinucleated giant cells. Proteins are either secreted factors (e.g. VEGF), membrane-associated proteins (e.g. RANK), or nuclear/cytoplasmic proteins (e.g. Runx2). Those proteins written in italics denote occasional expression by that cell type. Adopted from Robert *et al*., 2013.

2.1.4 Clinical Presentation

The main clinical symptoms are non-specific, local swelling, warmth, and pain radiating independently of weight bearing (Szendroi, 2004). Even more rarely, the disease is discovered radiographically in the absence of symptoms.

Pain may be activity related or experienced at rest or at night. Activity related pain is caused by the loss of structurally important bone and mechanical failure of bone as the result of the presence of the tumor. Pain at rest or at night is the result of tumor growth, tumor-related expansion of the periosteum, and the response of the periosteum to the threat of the advancing neoplasm. The duration of symptoms varies between two to six months and by then, in one-third of cases, the size of the tumour exceeds 50% of the diameter of the affected bone, it has destroyed the cortical bone and reached the subchondral region (Kevin *et al*., 2013). Pathological fracture is the first sign in approximately 15% of cases (Dreinfhofer *et al.,* 1995). GCT is usually a solitary lesion however 1-2% may be multicentric. The common sites of involvement are distal femur, proximal tibia and distal radius. Other less common sites are the sacrum, the distal tibia, proximal femur and proximal fibula. Involvement in the spine and sacrum, and the compression of nerve root by the tumour may cause intense radicular pain and neurological deficit.

Physical examination usually reveals an area of direct tenderness to palpation, soft tissue swelling over the affected area. The extension into the joint is unusual but if there is extension to the joint, it will lead to joint effusion, joint deformity and decreased range of motion. Patients often demonstrate an antalgic gait that favors the affected side.

GCT is usually a solitary lesion, however approximately 1% of cases present as multiple synchronous or metachronous lesions (K. F. Taylor *et al*., 2003). Most multifocal GCTs are synchronous and 68% of cases of multicentric giant cell tumor occur in less than 4 years from the initial lesion treatment (A. Haskell, 2003). They have a more aggressive course, including an increased incidence of pathologic fractures (B. W. Hindman, 1994)

In our centre, most of the patients presented late without any symptoms until aggressive breached of the cortex and may sometimes be dormant for a period of time (Faisham *et al*., 2006) consistent with findings by Ng *et al*., 2002 whereby 46% of their patients presented late with extensive involvement of soft tissue and articular surface and substantial proportion of patients seek traditional means of treatment before medical consultation.

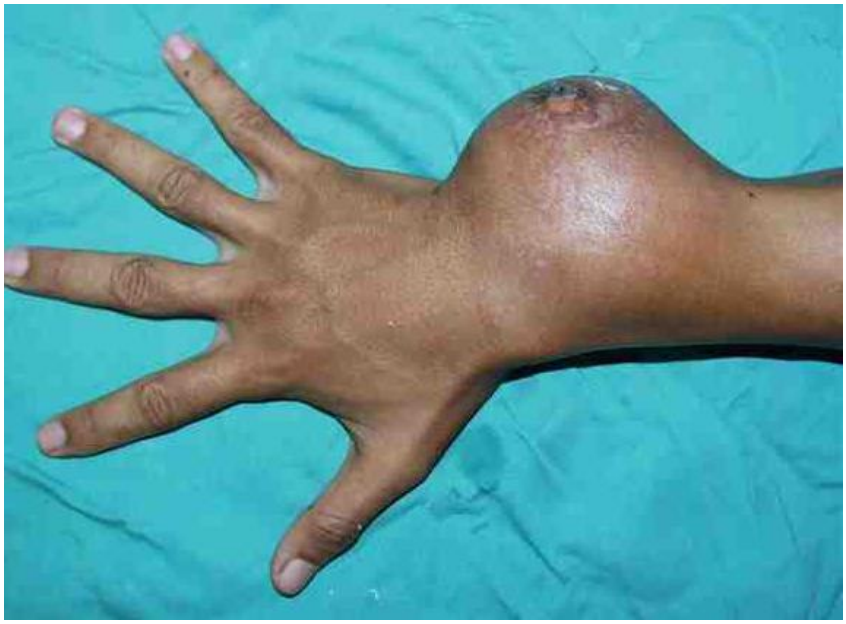

Plate 2 : Photograph of Patient With Giant Cell Tumour of Distal Radius

2.1.5 Radiographic Appearance

The typical appearance of GCT is best demonstrated on conventional radiographs which show a lytic lesion located at epiphyseal region of long tubular bones with a preference for the distal femur and proximal tibia, that has a well defined but non sclerotic margin, is eccentric in location, extends to the subchondral bone, and occurs in patients with closed physes (Murphey *et al.,* 2001; Turcotte *et al.,* 2006, Arnold *et al.,* 2011). There is no mineralized tumour matrix.

GCT may also have aggressive features, such as a wide zone of transition, cortical thinning, expansile remodeling, or even cortical bone destruction and an associated soft-tissue mass (Murphey *et al.,* 2001). These features may be more common in small-caliber long bones, such as the fibula or ulna, and pathologic fracture or periosteal reaction may occasionally complicate the diagnosis (Murphey *et al*., 2001).

Although GCT is typically included in the differential diagnosis for an epiphyseal lesion, there is evidence that it arises in the metaphysis and extends into the epiphyseal region after physeal closure (Campanacci *et al.,* 1987, Mirra 1989). For instance, in rare cases affecting pediatric patients, the tumor is centered within the metaphyseal region. In cases of multifocal GCT, lesions may occur in the metaphysis or even the diaphysis. There are no reported cases in which GCT has extended from the metaphysis into the epiphysis across an unfused physis (Murphey *et al*., 2001)

The radiographic features of GCT at sites other than the long bones are nonspecific and not unlike those of other osteolytic processes. Giant cell tumor of the spine almost always

begins in the vertebral body and may lead to vertebral collapse or extend into the intervertebral disc, adjacent vertebral body, spinal canal or paraspinal soft tissues (Dahlin *et al*., 1997) Sternal and sacral lesions are osteolytic and owing to a large size and a soft tissue component, may simulate the appearance of a malignant neoplasm. In the sacrum, the eccentric location and abutting of the SI joint differentiate GCT from similar appearing sacral chordomas. In the sacrum transarticular extension of the tumor may be noted.

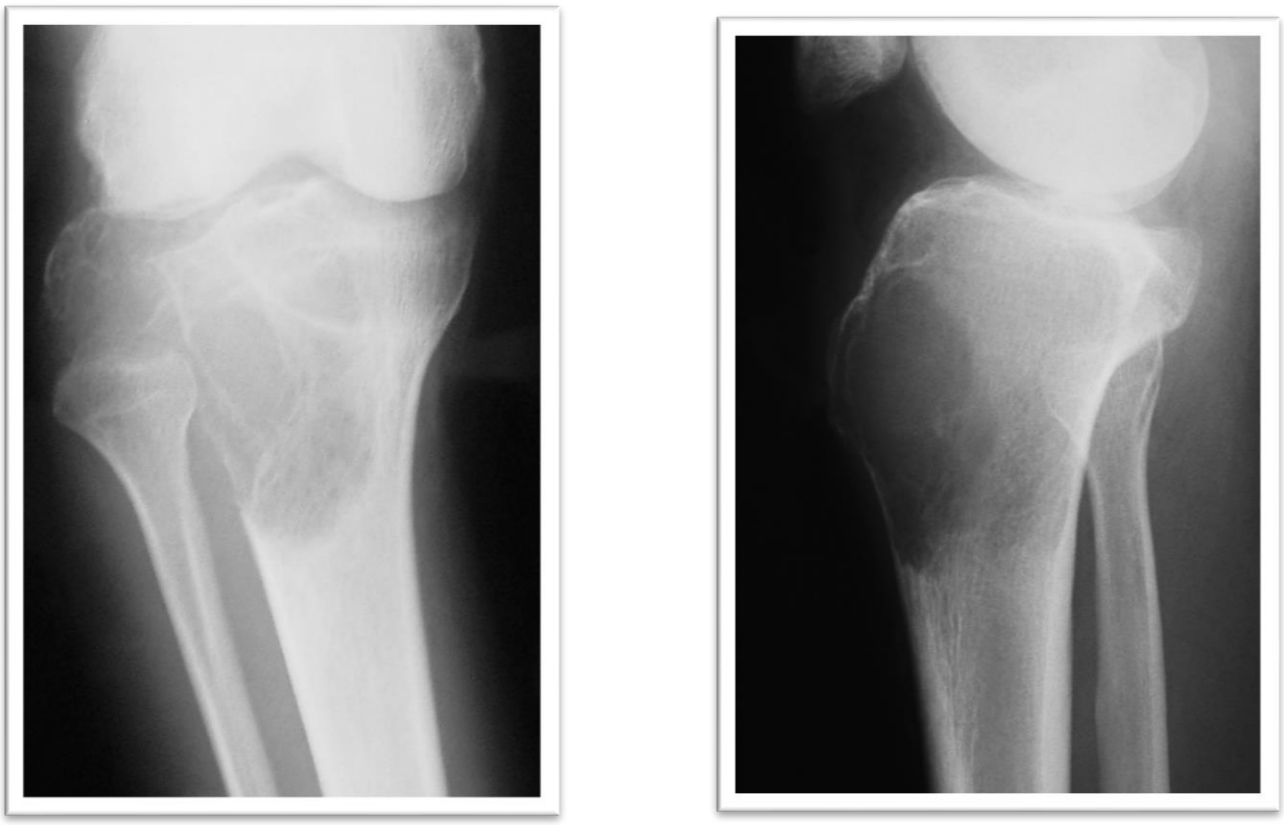

Plate 3: Radiographic Image of Giant Cell Tumour of Proximal Tibia, which describe expansile lytic lesion over metaphyseal area with subchondral extension and cortical breach.

2.1.6 Computed Tomography

Plain radiographs remain the mainstay of the diagnosis of GCT, however, MRI and CT are important for staging and therefore surgical planning. CT will rarely add additional information that changes the differential diagnosis (Moser *et al.,* 1990). However, CT is superior to conventional radiography and tomography in outlining tumor extent, especially its extra-osseous portion and its relationship to adjacent structures, as well as evaluation of cortical integrity and determination of tumor recurrence (Hudson *et al*., 1984). The expanded and thinned cortex is vividly demonstrated and the presence or absence of matrix calcification can be assessed. Fluid levels may be seen (Resnik *et al*., 1986) secondary to an aneurysmal bone cyst component or due to intratumoral hemorrhage. Reactive changes and edema on the outer cortical surface or the synovium may mimic tumor extension. The axial slices provided by CT do not allow accurate evaluation of the subarticular cortex because of volume averaging.

The advent of color volume rendered three-dimensional (3D) CT with video files allows evaluation of multiple tissues at the same time. The spatial depiction of the tumor along with surrounding anatomical relationships such as vessels and ureter make this a useful preoperative imaging modality in cases of pelvic GCT. Manipulation and rotation of the 3D images through 360 degrees allows the surgeon a better understanding of the extent of the mass and anticipated surgical complexities (Shaligram *et al.,* 2007).

Although CT angiography is seldom used as a diagnostic modality in the era MRI, it can determine the extra- osseous extent of tumour and its relationship to major vessels. The

role of angiography today, in patients with GCT is limited to a study of regional vascular anatomy and perhaps, preoperative transcatheter arterial embolization to facilitate excision and decrease surgical blood loss or in instance of unresectable neoplasm (Shaligram et al., 2007; Chuang et al., 1981).

2.1.7 Magnetic Resonance Imaging

MRI is currently the best imaging modality for GCT because of its superior contrast resolution and multiplanar imaging capabilities that allow accurate tumor delineation (Moser *et al.,* 1990). MRI is useful in determining extraosseous extent and articular surface involvement (Hermann *et al*., 1987), however subtle cortical destruction is better demonstrated by CT. MRI is also useful in assessing intraosseous and intramedullary skip lesions GCT shows low intensity on T1 and heterogeneous high intensity on T2 weighted images. Therefore intramedullary tumor is best seen on T1W, while its extraosseous portion is best appreciated on T2W images (Brady *et al.,* 1982)

The hypervascular stroma contains sinusoidal vessels that predispose to hemorrhage (Aoki *et al*., 1991). The phagocytosed erythrocytes lead to iron deposition in the form of hemosiderin (Aoki *et al.,* 1991). Giant cell tumors often have extensive hemosiderin deposition within tumor tissue, resulting in very low signal intensity on all pulse sequences (Aoki *et al.,* 1991). This is seen in up to 60% of cases (Aoki *et al.,* 1991). Low signal areas may also be due to collagen deposition secondary to surgery or trauma (Aoki *et al*., 1991). Gadolinium enhancement reveals areas of hypervascularity and enhancement with a very heterogeneous signal pattern (Resnik *et al.,* 1988).

MRI become important tool to detect giant cell tumour which undergoes malignant spontaneous transformation and to evaluate tumour which is located in the spine and sacrum where it helps to detect soft tissue extension such as compression to the spinal cord and nerve root (Turcotte *et al.*, 1990; Randall, 2003). Besides that, MRI is the optimum technique for evaluation of recurrent or residual disease. Local postoperative high signal within the surgical bed that exhibits a rounded mass-like appearance with eccentric growth is highly suggestive of tumor (Lee *et al*., 1998).

Magnetic resonance angiography (MRA), a newer and noninvasive technique, is used in addition to preoperative magnetic resonance imaging. Magnetic resonance angiography has the potential to replace conventional angiography in preoperative evaluation of upper-extremity tumours (Swan *et al.*, 1993). The anatomy represented gives information equivalent to conventional angiography. It provides a complete preoperative evaluation of a tumour bed or donor site for a vascularized graft harvest (Swan *et al*., 1993).

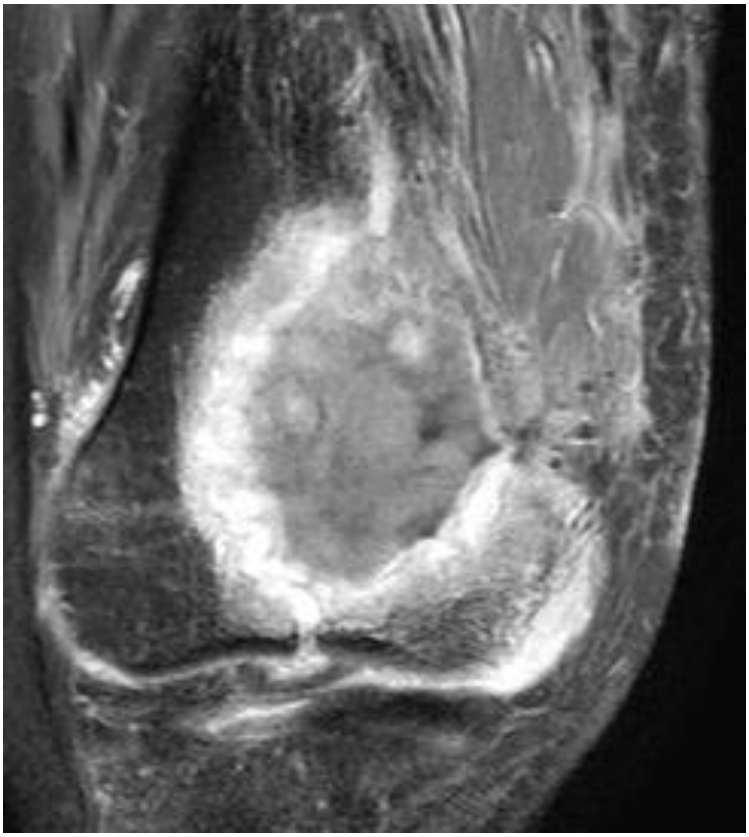

Plate 4 : MRI Image of Aggressive Giant Cell Tumour of Distal Femur showing articular and soft tissue extension.

2.1.8 CT Thorax

CT Thorax is important for the staging of GCT particularly screening for pulmonary metastases. It is also used as a baseline for subsequent comparison in search for pulmonary metastases (McKenzie, 1997).

2.1.9 Radionuclide Scintigraphy (Bone Scan)

GCT produces increased uptake of technitium-99m radiopharmaceuticals previously used to stage the GCT based on Campannaci classification. Bone scan can identify the latent, active and aggressive based on hot spot on bone scan. The pattern of increased uptake may be diffuse (40%) or peripheral with little central activity (60%) (Van et al., 1986). Extended patterns of radioactivity uptake beyond the margins of the tumor preclude accurate definition of intramedullary extent (Hudson et al., 1984). Increase uptake in the bone across the adjacent joint and in the joints of the same extremity not involved by tumour may occur (Gudmundsson et al., 1984). Therefore the role of bone scan in GCT is limited because it is nonspecific and unreliable in defining the extent of the tumour (Van et al., 1986). It is however, helpful in evaluating the rare patient with multicentric or metastatic GCT.

2.1.10 Gross Pathology

GCT of the bone involved epiphyseal-metaphyseal region of long bones (Goldring *et al.*, 1987). Grossly, the tumour is grey to reddish brown in colour and it is composed of soft, vascular and friable tissue. The unaltered lesional tissue appears rather homogeneous, with a tan colour and a moderately firm consistency. Firmer grey-yellow areas of fibrous and collagenization and osteoid production may be found as a result of previous fracture and degeneration. However, foci of hemorrhage or necrosis may be observed in many tumours. The overlying cortex usually undergoes resorption and the contour of the bone is expanded by the tumour which is covered by thin shell of subperiosteal new bone (Goldring *et al.*, 1987).

2.1.10 Histology

Giant cell tumor consists of three cell types: two mononuclear cells and the multinucleated, osteoclast like giant cells. The mononuclear cells are composed of a round and a spindle shaped cell type. The round cells have an oval nucleus with a central, prominent nucleolus. The spindle-shaped, fibroblast-like cells are characterized by a longitudinal often cigar-shaped nucleus. In some areas of the tumor the spindle-shaped cells are more prominent, showing a scattered fibrous background or storiform pattern. In these areas a reduced number of giant cells can be observed (Mirra 1989).

The number of multinucleated giant cells varies between different cases. In addition, the number of nuclei within the giant cells shows a broad variation from 5 to over 100 nuclei

(Delling 1997). The nuclei seemed to be identical to those in the round to oval cell types (Mirra 1989; Unni 1996). Mitotic figures are never seen in the giant cells. Presence of atypical mitoses may suggest malignant transformation.

Regressive changes like fibrosis, foam cell formations, and hemosiderin depositions can also be detected in some GCTs (Mirra 1989). Areas of infarct like necrosis are also common (Unni 1996). Occasionally, reactive bone formations as well as tumor osteoid formations are present (Mirra 1989). Various amounts of osteoid were found in 31% of the tumors, while reactive bone formations, especially in the periphery of the lesion, could be seen in 59% of the cases (M.Wulling *et al*., 2001). According to the local aggressive behavior of GCT, soft tissue extension is not an uncommon finding. In addition, multinucleated giant cells may be detectable within or penetrating the vascular lumina (Osaka *et al.*, 1997), but these findings do not seem to correlate with an increased risk of metastasis (Mirra 1989; Unni 1996). In 23% of tumors, giant cells were found in blood vessels (M. Wulling *et al.,* 2001). Metastasis of GCT occurs in 2% to 9% of large series (Bertoni *et al*. 1988; Kay *et al.* 1994).

Owing to the complex histological composition of GCT, differential diagnosis is required to exclude the diagnosis of other lesions also containing giant cells, such as variants of aneurysmal bone cysts, fibrous metaphyseal defects, chrondroblastoma, brown tumor in hyperparathyroidism, as well as giant-cell-rich variants of osteosarcoma.

To confirm the diagnosis, immunohistochemical analysis may be performed however it not part of routine investigation, only in highly suspicious of giant cell rich osteosarcoma and giant cell sarcoma. Giant cells constantly express CD68, an antigen expressed by

macrophages and osteoclasts, as well as p63. However, although sensitive to detection, p63 is not specific and can be expressed in other giant-cell-containing lesions of bone, specifically primary aneurysmal bone cysts, chondroblastomas, giant-cell reparative granulomas and some osteosarcomas (Doussis *et al*., 1992; Dickson *et al*., 2008).

2.1.11 Classification and Staging

Multiple classifications were develop to stage GCT. Jaffe et al 1940 grade GCT based on histological appearance of the stromal cells and the number of giants cells and mitoses, whereas Dahlin in 1949 distinguished only benign, aggressive and malignant. The histological staging system of Jaffe and its prognostic value of this grading had been disputed. Sanerkin *et al*., 1980 found no correlation between aggressiveness of the tumour and the histological grade. Thus, the prediction of the clinical behaviour of GCT based on its histological features is impossible.

Later on, Enneking 1986 and Campanacci 1987 classified GCT based on their clinical and radiographic features According to this system, there are three stages:

Stage 1: Benign, latent giant cell tumours. These lesions are characterized by a static pattern of growth, without features of local aggressiveness. They have well-margin border of a thin rim of mature bone. The cortex is intact or slightly thinned but nor deformed.

Stage 2: Benign, active giant cell tumours. These lesions are often clinically symptomatic. Plain radio- graphs, bone scans, computerized tomographic scans and magnetic resonance images demonstrate expansile radiolucent lesions, which frequently