TRANSCRIPTION FACTOR EXPRESSION IN SELECTED GIANT CELL LESIONS OF THE JAWS

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DECLARATION

I, Belinda Kathleen Bunn declare that this research report is my own work. It is being submitted for the degree of Master of Dentistry in the branch of Oral Pathology to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at this or any other University.

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Signature of Candidate

November 2012

DEDICATION

This research project is dedicated to my dear husband Nelson Fernandes for his unwavering love, support and encouragement for which I am eternally grateful.

ABSTRACT

The giant cell lesions of the jaws are characterised histologically by scattered multinucleated giant cells (MNGCs) within a connective tissue stroma containing round and spindled mononuclear cells. Additional features include haemorrhage and haemosiderin deposition. Central giant cell granuloma (CGCG), peripheral giant cell granuloma (PGCG), cherubism and aneurysmal bone cyst (ABC) are thus encompassed by this term. The osteoclastic nature of the MNGCs within these lesions is well established.

Microphthalmia-associated transcription factor (Mitf) is essential in the terminal differentiation of osteoclasts, the abnormal expression of which, results in dysfunctional osteoclast activity. Transcription factor E3 (Tfe3) belongs to the same transcription factor subfamily and is capable of forming co-immunoprecipitates with Mitf to function in a synergistic manner. It is abundantly expressed in physiological osteoclasts. Both factors are crucial for gene regulation in osteoclastic bone resorption.

This study aimed to assess the expression of Mitf and Tfe3 within the stromal and MNGCs of the aforementioned giant cell lesions in order to enhance our understanding of the biological nature of these cells. The results showed positive nuclear staining within both the stromal and MNGCs in all four lesions with preferential expression noted in the MNGCs. This finding supports the concept of precursor stromal cell fusion. In addition, Mitf was consistently expressed at higher levels than Tfe3, in keeping with its reported principal role in the terminal differentiation process. The only exception to this was observed in ABC where Mitf and Tfe3 expression levels proved to be similar. It is thus apparent that the co-expression of Mitf and Tfe3 serves to confirm the osteoclast-like phenotype of the MNGCs within the giant cell lesions of the jaws.

The degree of expression does not, however, correlate with the clinical behaviour of these cells, an observation substantiated by the minimal osteolytic potential of PGCG.

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CHAPTER 1

1.0 INTRODUCTION

The non-neoplastic giant cell lesions occurring within the tooth-bearing areas of the jaws are reactive or reparative in nature and include central giant cell granuloma (CGCG), peripheral giant cell granuloma (PGCG), cherubism and aneurysmal bone cyst (ABC).[1] These lesions share similar histological features and are frequently indistinguishable microscopically. They are characterised by numerous multinucleated giant cells (MNGCs) distributed within fibrous stroma containing ovoid to round and spindled mononuclear cells. Areas of haemorrhage with associated haemosiderin deposition and occasional foci of osteoid and woven bone are also encountered. Similar features are observed in brown tumours of hyperparathyroidism and giant cell tumour of bone (GCT). The former usually presents with multiple synchronous sites of involvement and is confirmed on serum analysis of calcium, phosphate, alkaline phosphatase and parathyroid hormone.[2] The latter is a true neoplasm typically affecting long bones with distinct clinical and radiographic features.[3]

The MNGCs within the giant cell lesions have osteolytic potential and exhibit definitive phenotypic characteristics of osteoclasts.[4, 5, 6] The mononuclear stromal cells are heterogeneous with subsets of osteoclast-like precursors and osteoblast-like cells. The osteoblast-like stromal cell component supports the process of osteoclastogenesis and is responsible for intralesional bone formation.[4, 5, 7]

Treatment of CGCG, cherubism and ABC usually involves surgical curettage with large aggressive lesions requiring mandibular or maxillary resection. Even small lesions may necessitate dental extractions resulting in severe functional and cosmetic deformities.

The expression of Microphthalmia-associated transcription factor (Mitf) is essential for the terminal differentiation of osteoclasts.[8] Abnormal Mitf expression results in dysfunctional osteoclast activity and has been implicated in the cause of osteopetrosis.[9] Mitf belongs to a transcription factor family which includes Transcription factor E-3 (Tfe3).[10] Tfe3 is abundantly expressed in osteoclasts and forms co-immunoprecipitates with Mitf. Mitf and Tfe3 regulate the expression of a number of enzymes which are central to the bone resorbing ability of osteoclasts. Tartrate-resistant acid phosphatase (TRAP) and the proteinase cathepsin K are two of the important transcription targets of both Mitf and Tfe3.[11] Furthermore, genetic evidence confirms these transcription factors as essential for the modulation of several functionally important genes. Mitf and Tfe3 heterodimerisation allows for significant target gene activation, especially of the enzyme TRAP, more so than either working alone. Mitf and Tfe3 thus function synergistically in gene regulation.[12]

Assessment of Mitf and Tfe3 expression within the mononuclear stromal cells and MNGCs would not only confirm the nature of these cells but assist in elucidating the mechanism of formation. Additional analysis of these transcription factors and the genes that they regulate may be of use in identifying novel therapeutic targets for the non-surgical management of aggressive osteolytic lesions.

Mitf expression has been described in CGCG and ABC.[9] Mitf expression has not been documented in PGCG and cherubism. Tfe3 expression, to the best of our knowledge, has not been assessed in any of the four lesions included in this study.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Giant cell lesions of the jaws

The pathogenesis of CGCG, PGCG, cherubism and ABC remains widely disputed, although lesions are generally accepted to be reactive and non-neoplastic. A proportion of these lesions are aggressive, exhibiting features more typical of a true neoplasm. Intrabony lesions are initially asymptomatic but may become extensive with widespread osteolysis precipitating pathological fracture. Occasionally, central lesions perforate the cortical plates to involve the overlying gingiva, presenting as peripheral lesions at first. Owing to the histological overlap of these lesions, clinicopathological correlation with radiographic features is always required to confirm the diagnosis.[2]

The exact histogenesis and cellular phenotype of the MNGCs has been the subject of extensive investigation. They have been suggested to represent a group of phagocytic cells in areas of haemorrhage due to the presence of intracytoplasmic haemosiderin fragments and vacuoles.[3] Alternatively, an endothelial or fibroblastic origin has also been proposed.[3, 5, 6] Owing to their prominence in some lesions, it has been suggested that they represent a population of giant tumour cells. Immunohistochemical staining with the proliferation markers PCNA and Ki67, however, fails to show evidence of mitotic activity within the giant cells. Strong nuclear signalling is conspicuous within the adjacent spindle-shaped stromal cells. As such, these cells are regarded as the true lesional cells and proliferative component whilst the MNGCs are accepted as secondary or reactive.[5, 13, 14] The MNGCs are now accepted to be osteoclasts.[4, 5, 6]

2.1.1 Central giant cell granuloma

CGCG (Figs. 1 and 2) is an osteolytic jaw lesion commonly encountered as a solitary, expansile intrabony mass of young patients.[15] Lesions occur more frequently in females under thirty years of age with preferential involvement of the mandible.[16] CGCG shows a distinct predilection for the tooth-bearing areas of the jaws, usually anterior to the first permanent molar teeth.[15, 16, 17, 18, 19] In addition, the lesions are most frequently encountered at the time of active tooth eruption. These lesions may therefore represent an abnormal localised proliferation of normal osteoclasts encountered during the phases of eruption and root resorption of the deciduous teeth.[2, 20] The diagnosis is often incidental as most lesions are asymptomatic with innocuous behaviour and favourable response to conservative surgical management. CGCG is generally accepted to represent a reparative or secondary disease phenomenon in response to intrabony haemorrhage or inflammation. It is also considered to form part of a spectrum of anomalous developmental lesions, pathogenetically related to ABC.[17] Prior to 1950, CGCG of the jaws was routinely diagnosed as GCT of bone.[21] The distinction between these two entities was first made by Jaffe [3] who proposed the term "giant cell reparative granuloma" for lesions of the jaws to emphasise their benignity.

A range of histological features and clinical behaviour has since been documented for CGCG.[16] For this reason, it is now deemed necessary to divide lesions into aggressive and non-aggressive types based on clinical and radiographic features.[21, 22] Non-aggressive lesions present as asymptomatic radiolucencies whilst aggressive behaviour is associated with pain, cortical bone destruction, resorption of adjacent tooth roots and increased tendency for recurrence. Age at presentation, lesion size, site of involvement and histology all influence the recurrence rate. Very young patients (mean = 10,7years) have the highest number of

recurrences whilst older patients (mean = 22,5years) are more likely to have non-aggressive lesions with no tendency to recur. Lesions greater than 3cm at presentation behave aggressively.[16] Lesional recurrence is site-dependent with those in the left mandibular ramus area recurring more often than lesions at any other site. Conversely, anterior maxillary involvement shows the lowest frequency of recurrence. A partial explanation for this finding is the presence of thin maxillary cortical plates which allow for rapid tumour expansion and earlier presentation. The anterior maxilla is also more likely to be radiographed at routine dental examination as compared to the posterior areas of the jaws, particularly the rami. Lesional proximity to the mandibular neurovascular bundle may not allow for adequate removal of lesional tissue, resulting in a high probability of recurrence.[21]

Several studies have attempted to correlate histological features with the clinical behaviour of these lesions. Statistically significant differences in the number of giant cells and their fractional surface area have been reported in aggressive and non-aggressive lesions. Aggressive lesions contain larger, more numerous giant cells compared to non-aggressive lesions in which the giant cells are smaller, irregularly shaped and fewer in number.[22] Osteoid production is reactive and is concentrated at the periphery of the lesional tissue where it separates lesional from non-lesional tissue, aiding surgical removal. Aggressive lesions are thus frequently devoid of osteoid production.

CGCGs are usually solitary, unilocular radiolucencies with a small percentage of multilocular lesions. Multilocular lesions are usually greater than 5cm in size, suggesting that multilocularity be regarded as an indicator of potentially aggressive behaviour. CGCG causes tooth displacement more readily than root resorption.[23] The radiological differential diagnosis includes an odontogenic keratocyst, ameloblastoma, odontogenic myxoma or ABC.[24] Large lesions cross

the midline to involve both sides of the jaw. The presence of multiple intrabony giant cell lesions is strongly suggestive of underlying hyperparathyroidism which requires clinical and serological exclusion.[25] Synchronous or multifocal giant cell lesions are exceedingly rare. Such apparent multifocality may be the result of haematogenous seeding or tumour spillage at the time of surgical removal. The term "craniofacial giant cell dysplasia" has been proposed to designate cases with true multifocality.[26] Bilateral lesions, particularly within the mandibular rami should raise the possibility of cherubism.[21] Synchronous giant cell lesions are more strongly indicative of multifocality whilst metachronous lesions may possibly represent recurrence due to seeding or incomplete excision.[24] Multiple cherubism-like central giant cell lesions have been described in the setting of a Noonan-like phenotype, for which an underlying genetic aetiology is implicated.[27]

There is no evidence of lesional regression. Untreated cases continue to enlarge with substantial facial distortion and dysfunction. The anatomical complexity of the maxilla and adjacent bones complicates simple curettage procedures. Maxillary lesions are associated with widespread sinus involvement, the thin cortical plates of which, precludes complete removal. Non-surgical management of such lesions is thus a desirable treatment modality. Intra-operative haemorrhage is a possible surgical complication due to the rich vascularity common to these lesions.[15]

Adjuvant radiation therapy has limited applications in the treatment of multiple recurrences and non-resectable lesions due to the risk of post-irradiation sarcoma development.[28] Calcitonin has been used effectively with dramatic clinical results and almost complete remission as an alternative non-surgical modality in several cases.[2, 29, 30] Additional benefits include the retention of teeth and undisturbed eruption patterns in younger patients. The duration of calcitonin

treatment is, however, protracted. Surgical excision therefore remains the best treatment modality for small, unilocular lesions.

Intralesional corticosteroid therapy has shown variable success with most lesions responding to a six week regimen. The mode of action in these cases is poorly understood. Small numbers of aggressive lesions fail to respond and continue to enlarge. Corticosteroids decrease the production of bone resorbing enzymes whilst inducing apoptosis of the osteoclastic giant cells.[13]

CGCG has been considered a proliferative vascular lesion due to its inherent vascularity.[31] Demonstration of low angiogenic potential within these lesions by means of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) expression, has led to the rejection of this theory.[32] Recent studies have, however, confirmed the osteoclastogenic role of VEGF within these lesions including those that are relatively avascular.[33] The primary mode of action of the anti-angiogenic agent interferon is the promotion of osteoblast differentiation from fibroblast precursors which in turn, stimulates bone production within these lesions as opposed to inhibition of angiogenesis.[13] A combination of interferon and conservative surgical debulking is therefore suggested. The significance and contribution of each treatment modality is difficult to assess in such cases, whilst the side effects of interferon prevent it from being used routinely.[34, 35, 36]

Figure 1: *The histological features of central giant cell granuloma are depicted in the photomicrograph below. Multinucleated giant cells are distributed in fibrous stroma containing mononuclear stromal cells, areas of haemorrhage, haemosiderin and admixed inflammatory cells. (H&E 2µm section; Original magnification: 200X).*

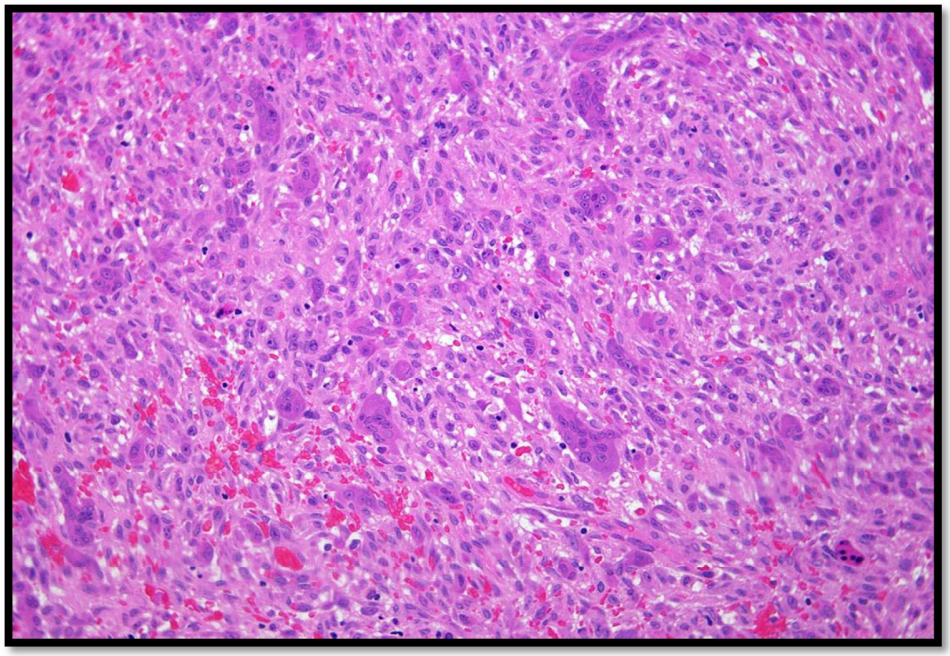
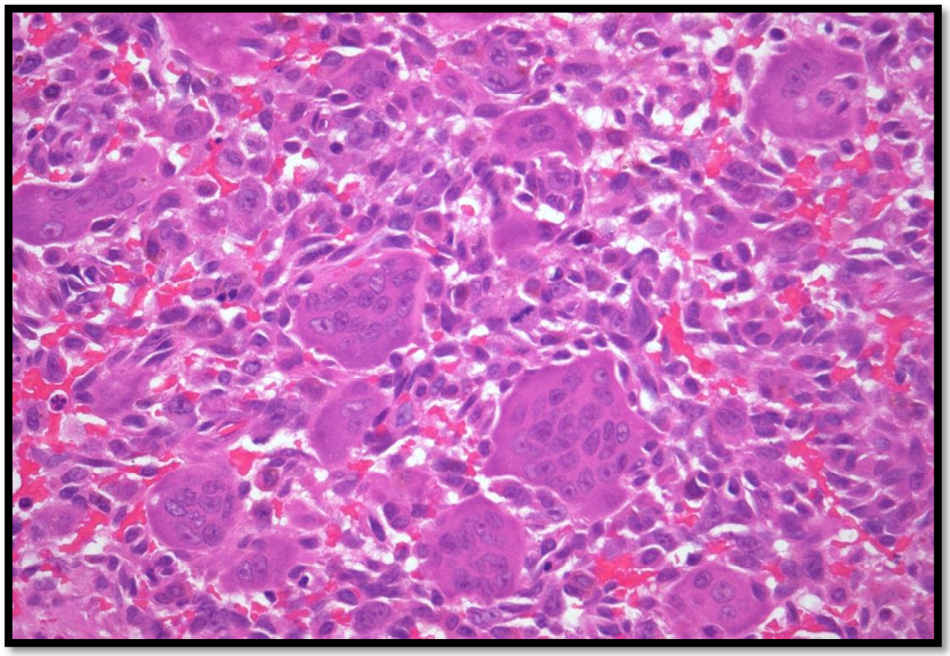


Figure 2: *The histological features of central giant cell granuloma are highlighted in the high magnification photomicrograph below. A mitotic figure is present centrally. (H&E 2µm section; Original magnification: 400X).*



2.1.2 Peripheral giant cell granuloma

PGCG is a hyperplastic, reactive lesion originating from the periosteum or periodontal ligament in response to chronic local trauma or irritation.[37] It is the most common of the four giant cell lesions in this study and has virtually identical histological features to CGCG, considered by some, to represent the soft tissue counterpart of CGCG. Lesions present as soft, polypoid gingival projections, varying in colour from dark purple to bright red (Fig. 3). Surface ulceration is frequently seen. The clinical differential diagnosis includes a pyogenic granuloma, fibrous epulis, peripheral ossifying fibroma, peripheral odontogenic fibroma, inflammatory fibrous hyperplasia, squamous papilloma, haemangioma or fibroepithelial polyp. Bony involvement by these lesions is distinctly uncommon; however, slight surface erosion may occasionally be seen. Widening of the periodontal ligament space of an adjacent tooth has been described.[14, 38]

PGCGs occur at any age, with most lesions reported in younger females. The maxilla and mandible appear to be involved with equal frequency, the most common site of involvement being the premolar-molar region. Microscopic examination confirms origin from the periodontal ligament, often demonstrating focal attachment. PGCGs are non-encapsulated lesions containing osteoclast-like MNGCs within a background stromal component of mononuclear cells (Fig. 4). Small areas of osteoid and woven bone may be observed (Fig. 5). The giant cells are frequently much larger than normal osteoclasts but do not typically show evidence of bone-resorptive function.[38] MB1 immunoreactivity has been demonstrated within the MNGCs in PGCG, confirming their osteoclast-like phenotype.[39] Little is understood regarding the activation or recruitment of the MNGCs in PGCG. Lesions often develop in areas where deciduous teeth have been replaced. This involves abnormal local proliferation of osteoclasts to allow for resorption

of roots and surrounding bone in the process of continued tooth eruption which may account for the formation of gingival masses.[40]

Brown tumours of primary or secondary hyperparathyroidism may rarely present as PGCG-like lesions. In such instances, a central brown tumour breaks through the cortical bone to involve the gingiva. Clinicopathological correlation with the radiographic features is required to rule out central origin. In addition, serological confirmation is required.[2, 40]

Complete surgical excision down to the involved mucoperiosteum is required to prevent recurrence. Removal of any adjacent causative factors or irritants is also advised. Recurrence has been described in about ten percent of cases. Several aggressive PGCGs occurring in young children have been documented. These lesions are associated with a greater degree of bone resorption of the interproximal crest area with displacement of the adjacent teeth and multiple recurrences.[38]

A recent study proposes that development of PGCG may be influenced to a certain extent by the presence of cytomegalovirus or Epstein-Barr virus. A positive association between the herpes virus family and widespread granulomatous lesions has been documented, hence the investigation of a causal relationship.[41]

Figure 3: *The photomicrograph below shows a low power view of a peripheral giant cell granuloma with intact overlying oral epithelium on the left. (H&E 2µm section; Original magnification: 100X).*

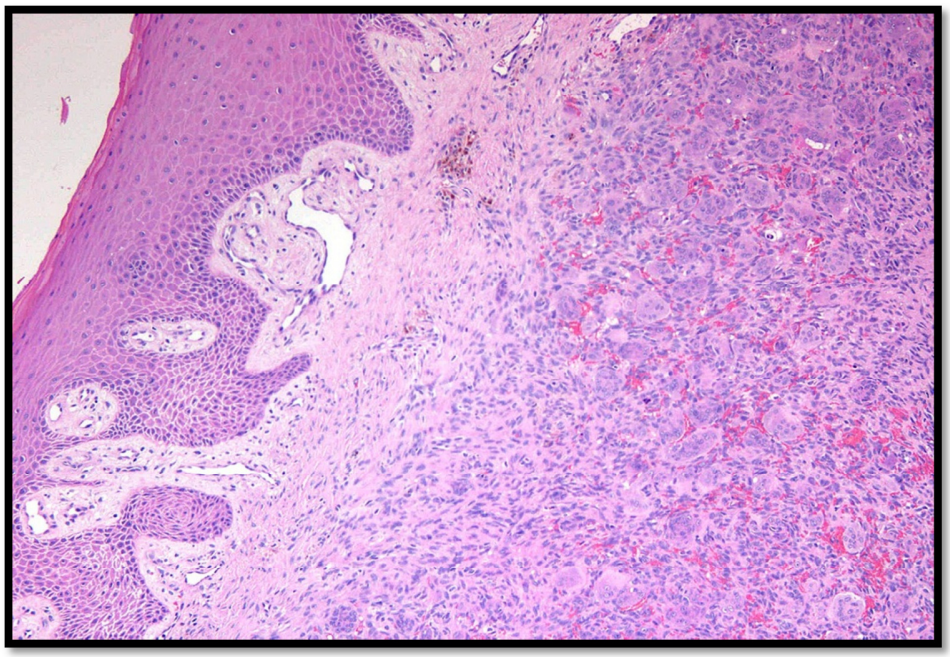


Figure 4: *The photomicrograph below shows the histological features of peripheral giant cell granuloma at higher magnification. Large osteoclast-like giant cells are distributed within cellular fibrous stroma containing mononuclear stromal cells. Areas of haemorrhage are present. (H&E 2µm section; Original magnification: 200X).*

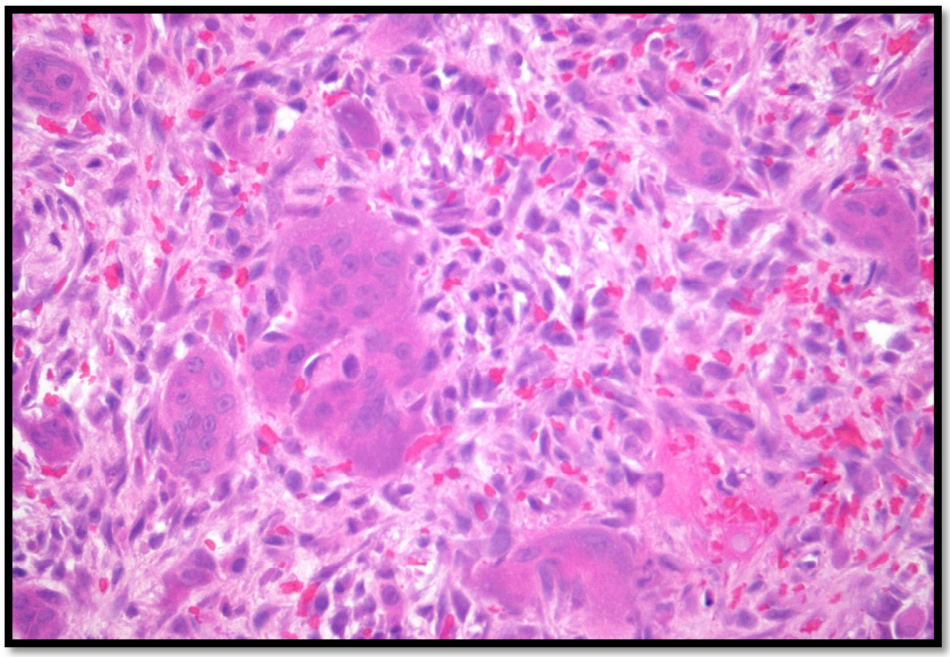
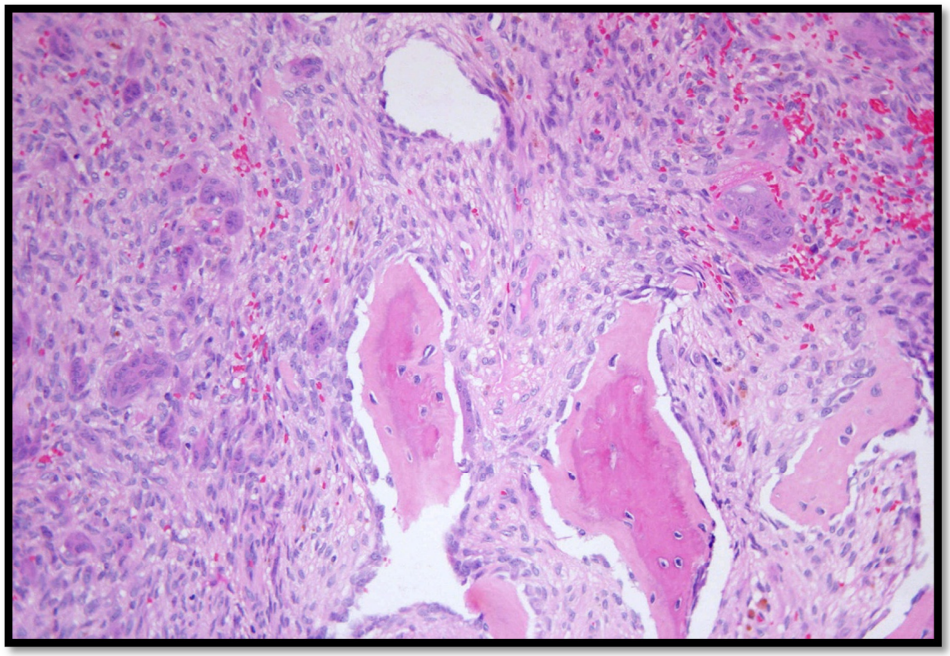


Figure 5: *The photomicrograph below shows a peripheral giant cell granuloma with areas of osteoid and woven bone. The immature bone is reactive in nature and is commonly seen at the periphery of the lesional tissue. (H&E 2µm section; Original magnification: 200X).*



2.1.3 Cherubism

Cherubism (Figs. 6&7), was first described as "familial multilocular cystic lesion of the jaw" in 1933.[42] It is a rare hereditary giant cell lesion regarded by some as a specific form of fibrous dysplasia. The World Health Organisation defines cherubism as a non-neoplastic bone lesion affecting only the jaws.[43]

Mutations in the SH3BP2 gene have been documented in several cases of cherubism, suggesting an underlying genetic aetiology. The disorder exhibits an autosomal dominant pattern of inheritance with complete penetrance in males and incomplete penetrance in females and variable expressivity.[43-47]

Cherubism is characterised by bilateral, symmetrical maxillary or mandibular enlargement. The variable phenotypic expression results in an absence of clinical disease in the mildest forms. The

most severe cases present with marked enlargement of the maxillary and mandibular bones as well as respiratory, visual, speech and swallowing difficulties. Affected patients present early, between the ages of two and five years of age. The jaw lesions are slowly progressive until puberty at which time they stabilise with regression thereafter. By early adulthood, the regression results in minimal residual deformity. Radiographic examination shows bilateral multilocular areas within the posterior mandible or maxilla. Dental abnormalities including congenitally missing teeth, premature exfoliation of primary teeth and displacement of permanent teeth are common. Lesions often coincide with the development of the second and third molar teeth. The dental abnormalities seen in these patients, suggests that the SH3BP2 gene is linked to tooth development and eruption.[43] It has thus been suggested that cherubism be defined as a "genetically determined alteration of tooth development".[44]

The SH3BP2 gene is mapped to chromosome 4p16.3 where it encodes an adaptor protein regulating the transcription factor nuclear activator of T-cells (NFAT) which plays a role in osteoclastogenesis.[45] Knock-in murine models with the most common human SH3BP2 mutations, show activation of tumour necrosis factor-alpha (TNF-α) expression by myeloid cells which results in bone loss and inflammation.[46] Point mutations have been identified in exon 9 of this gene with amino acid substitutions within the six amino acid sequence from position 415-420.[43, 47] Recently, a similar mutation was identified in a single case of CGCG but not in PGCG. All three lesion types, however, show increased expression of the c1 isoform of NFAT. RANKL expression has also been shown to stimulate SH3BP2 resulting in the up-regulation of NFATc1 in bone marrow during osteoclast differentiation. NFATc1 regulates the expression of several important osteoclast-specific enzymes whilst increasing osteoclast fusion.[48] Molecular testing is useful for the identification of individuals at familiar risk. In addition, genetic counselling may

be required as each child born to an individual with cherubism has a fifty percent chance of inheriting the same mutation.[45]

Noonan-like/multiple giant cell lesion syndrome is a rare condition characterised by dysmorphic features, developmental delay, short stature, pulmonary stenosis and multiple giant cell lesions of the bones and soft tissue. It is associated with mutations in the PTPN11 gene. The condition is easily mistaken and often misdiagnosed as cherubism due to the overlapping clinical and histological features. Cherubism has, however, been described to occur as a component of Ramon syndrome in which patients have a short stature, intellectual disability and generalised gingival fibromatosis.[45]

Treatment by means of curettage and bone grafting is performed in only the most aggressive, destructive lesions which have caused disfigurement. Patients often require adjunctive orthodontic therapy and in severe cases, ophthalmologic treatment for visual loss or globe displacement.[45]

Figure 6: *The histological features of cherubism are depicted in the photomicrograph below. Numerous multinucleated giant cells, some of which are irregularly shaped, are distributed in fibrotic stroma containing mononuclear stromal cells, extravasational haemorrhage and inflammatory cells. (H&E 2µm section; Original magnification: 200X).*

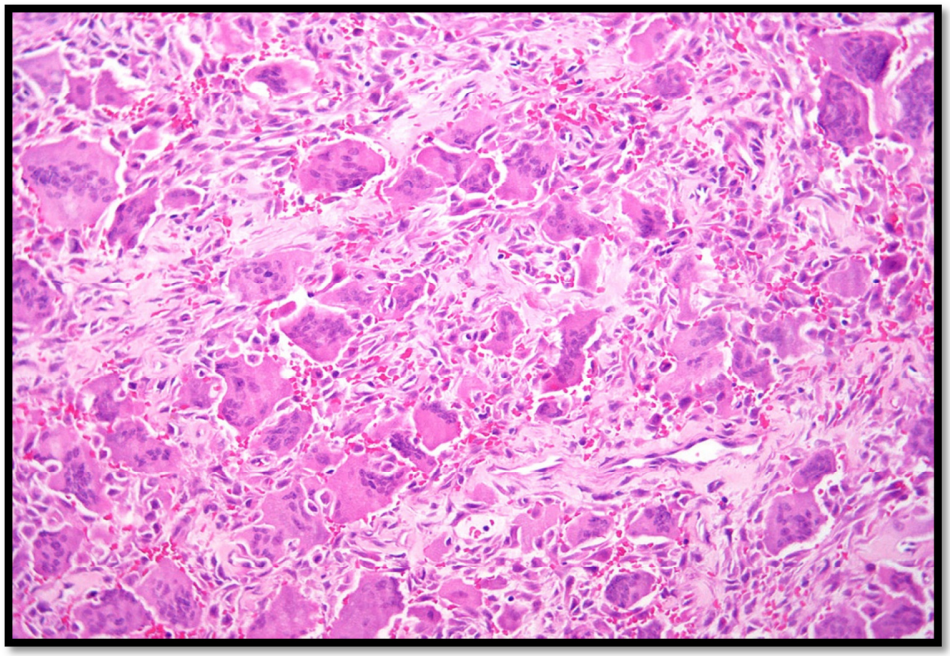
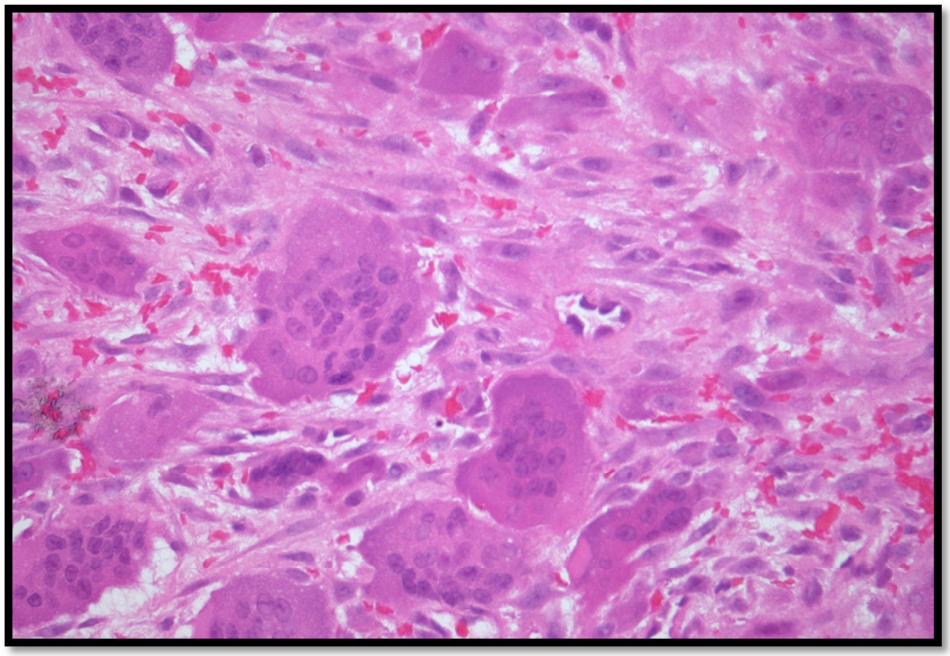


Figure 7: *The histological features of cherubism are highlighted in the high power photomicrograph which follows. (H&E 2µm section; Original magnification: 400X).*



2.1.4 Aneurysmal bone cyst

ABC is a pseudocystic, reactive giant cell lesion characterised by large blood-filled sinusoidal spaces which cause ballooning of the cortical bone. ABCs have a predilection for bones with both high venous pressure and marrow content. The bones of the craniofacial skeleton have low venous pressure which accounts for the rarity of ABCs at this site.[49] ABCs of the jaws account for only 1,9% of all skeletal ABCs.[50]

ABCs occur in patients younger than twenty years of age with no distinct gender predilection. The mandible is involved three times as often as the maxilla and most frequently in the molar area. Lesions present as firm, asymptomatic bony swellings which progressively enlarge with resultant cortical expansion and rarely perforation. Larger lesions may cause slight pain and tenderness. The associated teeth are vital and may be displaced by the enlarging lesion.[50, 51]

The non-specific radiographic features include the presence of a well demarcated, multilocular radiolucent lesion, often described as having a "honey-comb" or "soap bubble" appearance. An accompanying periosteal reaction is documented in aggressive lesions. Resorption of adjacent tooth roots is rare.[51]

ABC histology is characteristic of giant cell lesions, although the stroma is more fibrogenic. In addition, large pseudocystic, cavernous blood-filled spaces are conspicuous (Fig. 8). "Solid" variants of ABC have been described, overlapping to some extent with CGCG. The giant cells in ABC are generally associated with the physiological processes of resorption and repair.[49] ABC was initially suggested to be a secondary phenomenon occurring within a pre-existing lesion, the features of which are obscured by extensive tissue destruction and haemorrhage.[52] ABCs have been documented in association with ossifying fibroma, chondroblastoma, GCT, osteoblastoma,

CGCG, fibrous dysplasia, odontogenic myxoma and solitary bone cyst.[49] In such cases, cellular oedema results in stromal microcyst formation. The microcysts subsequently enlarge due to stromal collapse. Osmotic forces rupture regional thin-walled blood vessels with resultant intraluminal haemorrhage.[49, 53] ABC may truly co-exist with CGCG whilst CGCG may evolve into an ABC at some stage.[49]

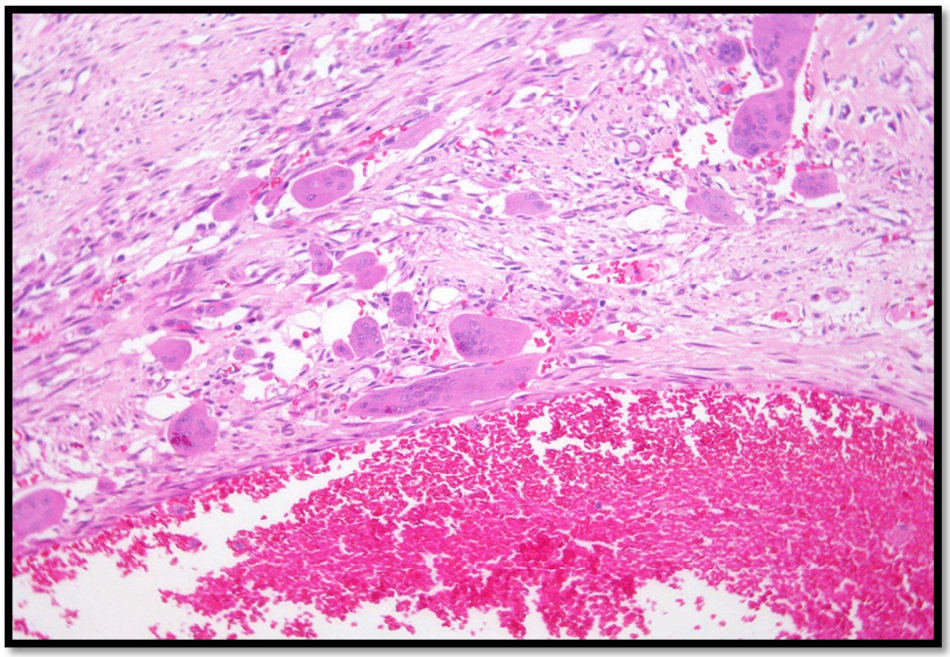
Aspiration of blood in the absence of a thrill, bruit or pulse pressure are useful adjuncts to clinically distinguish ABC from other vascular lesions. Computerised tomography (CT) scans are diagnostically useful and show multiple fluid levels due to erythrocyte sedimentation.[49] Aggressive cases enlarge rapidly and destroy adjacent cortical bone. These lesions may be so large as to encompass the inferior alveolar canal and mimic a malignant process. ABCs do not, however, cause root resorption or paraesthesia. An osteosarcoma should be ruled out in cases presenting with clinical features suggestive of a malignant process. This is achieved by histological appreciation of stromal anaplasia and mitotic activity. It must be borne in mind that an ABC may be superimposed on an osteosarcoma, with malignant areas not being represented microscopically.[49-52]

The exact aetiology and pathogenesis of ABCs is yet to be resolved. Patients often provide a history of preceding trauma to the area, although the underlying causal relationship with ABC formation is not well understood. ABC, CGCG and simple bone cyst have all been theorised to arise due to a vascular defect. Trauma or an aneurysm may lead to the formation of an intramedullary haematoma. If the haematoma is in direct contact with a large blood vessel, the increase in blood pressure will result in ABC formation.[54, 55]

ABC is suggested to represent a vascular abnormality resulting from persistent local haemodynamic changes. These changes lead to an increase in venous pressure with congestion and dilation of the vascular bed. The raised local blood pressure is thought to be a stimulus for bone resorption. The initial haemodynamic changes may be due to venous thrombosis or an arterio-venous aneurysm. ABC may represent an early canalising haematoma prior to organisation and related to CGCG. If damaged blood vessels remain in continuity with the haematoma, an ABC forms whilst CGCG forms if this continuity is lost.[51]

Conservative surgical excision is the treatment of choice for ABC. Radiation has been used but is not advocated due the attendant risks of sarcomatous change. Recurrences within the jaw and facial bones are rare and usually due to inadequate surgical access with incomplete excision.[50]

Figure 8: *The characteristic histological features of aneurysmal bone cyst are depicted in the photomicrograph below. A large blood-filled pseudocystic space is present inferiorly, surrounded by fibrous stroma containing scattered giant cells. The stroma is notably less cellular than in the other giant cell lesions. (H&E 2µm section; Original magnification: 200X).*



2.2 Giant cell phenotype and cellular interactions in giant cell lesions of the jaws

The MNGCs in the so-called giant cell lesions of the jaws closely resemble the giant cells typically encountered in granulomatous inflammatory and reactive lesions. These cells represent the fusion of histiocytes in response to indigestible or foreign material. The fusion process occurs when two or more epithelioid histiocytes attempt to phagocytose the same particle. The giant cells in chronic inflammatory lesions are derived from peripheral blood monocytes which are attracted to the site of inflammation by potent chemo-attractant agents, varying in size with up to one hundred nuclei per cell.[56]

Several theories regarding the origin of the MNGCs within the giant cell lesions of the jaws have been proposed. As they are concentrated in areas of haemorrhage with a tendency to form aggregates around blood vessels, it was suggested they be derived from circulating monocytes (of the macrophage-monocyte lineage) as a phagocytic response to extravasated red blood cells.[3] Endothelial cell origin in response to trauma or secondary vascular alterations is also suggested.[16] Several authors have considered them to represent foreign-body type cells.[5, 13] Recent literature shows a general consensus regarding the osteoclastic phenotype of the MNGCs which has been ultrastructurally, morphologically and immunohistochemically confirmed.[4, 5, 6, 57-60] Favourable response to calcitonin, a known osteoclast inhibitor, as well as the anti-osteoclast factors protegerin and human monoclonal antibody AMG 162 further support this.[2, 13]

Previous studies have focused on establishing the osteoclast phenotype of the MNGCs within the giant cell lesions of the jaws, thereby accounting for the osteolytic potential of these lesions. Vital cell culture and morphological examination of the giant cells confirms the presence of motile cells with abundant cytoplasm and broad pseudopodia.[4, 57] Furthermore, MNGCs cultured

on cortical bone and dentine slices are capable of active bone resorption. The addition of calcitonin to such cultures has an inhibitory effect characterised by cellular retraction and immobility.[4, 6] Occasional adjacent round mononuclear stromal cells are similarly inhibited.[4] Studies have demonstrated that the MNGCs as well as a small proportion of adjacent stromal cells consistently express osteoclast-specific antigens whilst producing enzymes typical of physiological osteoclasts.[4-7, 9, 57, 50-62] Enzyme histochemical analysis shows production of a variety of enzymes including TRAP, vacuolar H^+ ATP-ase (V-ATPase), carbonic anhydrase II (CAII), cathepsin K, matrix metalloproteinase-9 (MMP-9), vitronectin receptor (VNR), succinic dehydrogenase, cytochrome oxidase and β-glucoronidase by the MNGCs as produced by active physiological osteoclasts.[4, 5, 57, 59] These enzymes are all essential for bone demineralisation and collagen degradation which reinforces the osteolytic potential of these cells. A small proportion of the adjacent stromal cells produce a range of similar enzymes, lending support to the theory that these cells fuse to form the giant cells.[4, 5] The giant cells fail to show any alkaline phosphatase (ALP) production, an important marker of an osteoblastic phenotype. Adjacent spindled stromal cells do, however, abundantly express ALP, suggesting these cells are osteoblasts. In addition, the spindled cells show high proliferative activity with numerous PCNA-positive spindled stromal cells being identified in close proximity to newly deposited osteoid within these lesions, adding to the evidence of osteoblastic differentiation.[4]

Immunohistochemical assays have shown consistent giant cell immunoreactivity for CD68, thus confirming the macrophage/monocyte lineage of these cells.[4, 5, 58] CD68 is expressed by both macrophages and osteoclasts. The MNGCs within the giant cell lesions fail to express any of the macrophage-specific markers including CD11a, CD11b, CD14 and HLADR providing further evidence of an osteoclast phenotype.[4]

The relationship between the stromal and giant cells can be used to explain the osteoclastogenesis process within these lesions. The stromal cells are heterogeneous and comprise two morphologically and immunohistochemically distinct cell populations. The vast majority of cells are spindle-shaped and resemble fibroblasts whilst approximately ten percent of cells are plumper with rounded nuclear morphology and similar enzyme histochemical and immunohistochemical profile to the MNGCs. The latter are thus recognised as the giant cell precursors which fuse to form the osteoclast-like giant cells.[4, 5, 33, 61]

Whilst expressing ALP and elaborating osteoid, the spindled stromal cells also secrete the fibroblast related antibodies prolyl-4-hydroxylase and vimentin as well as a range of factors known to support the osteoclastogenesis process.[4. 5. 6] CD14-positive peripheral blood monocytes cultured with stromal cells in the presence of macrophage-colony stimulating factor (M-CSF) transform into mononuclear and multinuclear TRAP-, VNR+ cells to substantiate this.[4]

The receptor activator of NF-κB (RANKL) is essential for osteoclast formation, the role of which is discussed in more detail in the section on osteoclastogenesis which follows. The spindled stromal cells are strongly positive for RANKL expression by immunohistochemical and in-situ hybridisation studies.[5, 61] RANKL induces osteoclastogenesis by binding to RANK on the surface of the round mononuclear stromal cells. It is also capable of direct osteoclast stimulation. RANKL-induced osteoclastogenesis and osteoclast function are inhibited by Osteoprotegerin (OPG) which competitively binds to RANK. OPG expression is extensively distributed between both the stromal and giant cell components whilst the expression of RANK appears to be restricted to the stromal and occasional giant cells.[5] This differential cellular expression of RANKL, RANK and OPG further exemplifies the osteoclastic nature of the giant cells whilst sustaining the hypothesis of stromal cell fusion. The stimulatory and supportive role

of the spindled osteoblastic stromal cells in the osteoclastogenesis process is also confirmed.[4, 5, 62] Occasional spindled stromal cells which do not express RANKL are assumed to represent scattered mature fibroblasts.[62]

Mitf and Tfe3 are transcription factors which are essential for the terminal differentiation phase of osteoclastogenesis. Mitf expression has been demonstrated within the MNGCs of CGCG and ABC with occasional stromal cells showing similar immunoreactivity. This once again strengthens the principle of stromal cell fusion in the osteoclastogenesis process.[9]

In summary, the production of bone resorptive enzymes, expression of osteoclast-specific monoclonal antibodies, the ability to experimentally resorb bone as well as the therapeutic inhibition by calcitonin, are all features which provide definitive evidence of an osteoclast phenotype within the MNGCs of the giant cell lesions of the jaws.

2.3 Physiological osteoclasts and the process of osteoclastogenesis

Physiological osteoclasts are multinucleated giant cells found throughout the body. The cells are formed through a process of synchronous fusion of mononuclear cells belonging to the macrophage lineage and originating from the haemopoietic system.[33, 62] Osteoclasts are responsible for bone resorption, a process by which the mineral content is removed with subsequent breakdown of the organic component. Together with osteoblasts, osteoclasts are essential for the remodelling and maintenance of healthy bone.

2.3.1 Osteoclast structure and function

Osteoclasts have unique histological features which are best demonstrated by means of electron microscopy. Osteoclasts are most abundant immediately adjacent to or in very close association

with living, vital bone where they lie in resorption bays termed Howship's lacunae. The cell membrane is intricately folded to form a "brush border" or ruffled membrane which increases the surface area over which bone resorption can occur. Within the cytoplasm closest to the ruffled membrane, are abundant contractile and fibrillar proteins which are responsible for mediating attachment to a mineralised surface. The area of osteoclast attachment to bone is sealed off from the surrounding tissue and is referred to as the "sealing zone". This isolates a micro-environment between the osteoclast and the bone surface.[63] Osteoclasts bind to adjacent bone by means of membrane bound integrins. Aγβ3 is an example of such an integrin which typically binds to the extracellular matrix protein osteopontin to facilitate intracellular binding. The physical act of cell binding activates an adhesion kinase with resultant osteoclast activation.[64] Osteoclast activation brings about the formation of the aforementioned impermeable sealing zone.[65] Osteoclasts are approximately 40μm in diameter and have an average of fifteen to twenty closely packed nuclei. Each nucleus is surrounded by a well-developed golgi complex. The cytoplasm has a vacuolated, somewhat foamy appearance due to the presence of numerous cytoplasmic organelles which include mitochondria, free polysomes, rough endoplasmic reticulum, vacuoles and coated transport vesicles.[63, 65] An integral component of all osteoclasts is the presence of a proton pump. The proton pump is associated with the ruffled border and pumps hydrogen ions into the sealing zone, thereby creating an acidic micro-environment which supports the bone resorptive process. The main function of the acidic micro-environment is the demineralisation of bone in order to expose its organic matrix. At the same time, an optimal pH is created for the functioning of the proteolytic enzymes produced by the osteoclasts. The ruffled membrane previously described, is a characteristic morphological feature of activated osteoclasts which are in the process of resorbing adjacent bone. The hydroxyapatite mineral portion of bone includes

calcium and phosphate ions. The ions are absorbed into small membrane-bound vacuoles by the process of endocytosis. They are released into the extracellular fluid at a later stage accounting for the increased serum levels of calcium and phosphate.[65]

2.3.2 Osteoclastic proteolytic enzymes

The osteoclast produces several proteolytic enzymes which degrade the mineral and organic bone matrix by the bone resorptive process. These enzymes include the following:

i. Collagenases belonging to the matrix metalloproteinase family (MMP). MMP-1, MMP-8, MMP-9, MMP-13 and MMP-14 are some such examples. MMP-14 is also referred to as the membrane-type matrix metalloproteinase (MT-1MMP).[65]

ii. Members of the cysteine proteinase family which includes cathepsin B, cathepsin L and cathepsin K.[66]

iii. Tartrate-resistant acid phosphatase (TRAP). This enzyme is widely used as a specific histochemical marker of osteoclasts in bony tissue.[65] Expression of TRAP is a useful indicator of an osteoclast phenotype and distinguishes osteoclasts from other forms of giant cells and macrophages.[60, 63]

iv. Carbonic anhydrase II (CA II). This enzyme is expressed in high concentration, facilitating the action of carbonic acid, enabling the liberation of protons.[64]

v. Vacuolar H^+-ATPase (V-ATPase). V-ATPase forms an electrogenic pump involved exclusively in the transport of protons.[65]

Chloride channels on the osteoclast membrane allow for the balancing of charge and for passive chloride-bicarbonate exchange in order to maintain the intracellular pH of the osteoclast.[64, 65]

Mammalian osteoclasts have numerous specific binding sites for calcitonin. The expression of calcitonin receptors is therefore regarded as one of the most reliable markers of osteoclast differentiation. Calcitonin inhibits the functioning of osteoclasts by binding to the cell surface calcitonin receptors. This results in decreased bone resorption. Calcitonin further reduces the bone-resorptive process by its action on osteoclast progenitor cells.[2, 65]

The most functionally important osteoclastic enzyme is cathepsin K. Cathepsin K is a cysteine protease which has highly effective matrix degrading ability. It is predominantly expressed in osteoclasts and functions to destabilise the triple helix of fibrillar collagen which is the primary constituent of bone matrix. Following action by cathepsin K, collagenases and proteases are able to continue the collagen degradation process.[66] The cathepsin K gene has been mapped to chromosome 1q21. Cathepsin K deficiency, often due to gene mutation, results in a rare disorder known as pycnodysostosis. Physical features of this disease process include a short stature, acro-osteolysis of the distal phalanges and skull deformities.[67] Disease severity in deficient subjects highlights the functional significance of cathepsin K in bone homeostasis. Cathepsin K-deficient mice develop a severe osteosclerotic disorder with associated osteopetrosis, particularly of the long bones and vertebrae.[68] Osteoclasts deficient in cathepsin K are able to demineralise the extracellular matrix normally, however, degradation of the demineralised organic matrix is retarded. The co-expression of V-ATPase is essential to create the acidic environment which facilitates cathepsin K activity. The MNGCs in GCT of bone show marked cathepsin K expression. In addition, they also produce high levels of cathepsin L and MMP-9.[66] The function of cathepsin L is poorly understood with knockout mice developing periodic hair loss and epidermal hyperplasia but no signs of osteosclerosis. Furthermore, the MMP-9 produced by these cells is present in an inactive proform.[69] Cathepsin K is thus preferentially expressed and

is thought to account for the osteolytic nature of GCT.[66] Although it is a useful indicator of the functional status of an osteoclast, cathepsin K expression is not osteoclast-specific. It is widely expressed by various tissues and is therefore not useful for characterisation of the osteoclast phenotype. Increased expression of cathepsin K in human breast cancer is proposed to contribute to tumour invasiveness.[11] Cathepsin K expression has been reported in the ovary, within macrophages associated with atherosclerotic lesions as well as in several epithelial cell types in the kidney, cartilage and in hypertrophic chondrocytes.[64, 67] Its expression appears to be upregulated in lung tumours.[68] Assessment of cathepsin K expression in various granuloma-containing lesions within soft tissue including sarcoidosis, tuberculosis and foreign body giant cell reactions, showed positive expression by both the epithelioid histiocytes within these lesions as well as the MNGCs.[69] By contrast, normal resident macrophages, show no evidence of cathepsin K expression. The expression is limited to the epithelioid histiocytes and MNGCs and is thus proposed to represent a marker of macrophage differentiation. Cathepsin K expression is therefore useful for distinguishing between non-stimulated tissue macrophages and those that are highly active. The quantitative degree of cathepsin K expression may therefore also serve as a measure of immune efficiency in lesional tissue.[69]

The expression of cathepsin K is regulated by Mitf in osteoclasts. In addition, Mitf regulates cathepsin K expression in melanocytes and breast tumours. Concordantly, Mitf expression is thus integral for both osteoclast function and bone homeostasis.[11]

2.3.3 Osteoclastogenesis

Osteoclastogenesis (Fig. 9) involves proliferation of osteoclast progenitor cells which differentiate into mononuclear pre-osteoclasts. The pre-osteoclasts undergo a fusion process to

give rise to the characteristic multinucleated osteoclasts.[63, 65] In vivo experiments confirm the haemopoietic stem cell origin of osteoclasts.[65] The transcription factor Pu.1 acts on common myeloid progenitor cells and is crucial for the development of all early osteoclast precursor cells. It also functions to bring about development of lymphocyte and granulocyte precursors.[10] Under the influence of macrophage colony stimulating factor (M-CSF), early macrophage and osteoclast progenitors are stimulated to proliferate. Treatment of co-cultures with anti M-CSF antibodies or anti M-CSF receptor antibodies completely inhibits osteoclastogenesis during both the proliferative and differentiating phases.[70] The results obtained experimentally indicate that M-CSF is essential for both the proliferation of osteoclast progenitors as well as the differentiation process.

The osteoclast progenitor cells require the action of the transcription factors nuclear factor kappa B (NF-κB) 1 and 2 as well as that of c-Fos for further development and differentiation into committed osteoclast precursors. The osteoclast precursor cells do not yet express TRAP. NF-κB1 and 2 as well as c-Fos knockout mice, have a complete lack of osteoclasts and significantly increased numbers of macrophages. The absence of these factors arrests the differentiation of macrophages and osteoclast precursors. The outcome of this is severe osteopetrosis.[8, 10, 70]

Committed osteoclast progenitors are thus derived from immature haemopoietic stem cells. Peripheral blood monocytes and occasional macrophages are also capable of osteoclast differentiation, provided they are in the presence of suitable stromal cells which function to support osteoclast development[64, 65] Osteoblastic stromal cells are capable of such support. They are in fact an essential component in osteoclastogenesis, producing several factors which initiate osteoclastogenesis in osteoclast precursors or peripheral blood monocytes. Direct cell-cell contact appears to be a prerequisite for osteoclastogenesis to occur and facilitates the actual

fusion process. In vitro generation of osteoclasts has successfully been achieved by culturing both mouse and human monocytes together with osteoblasts or with stromal cells derived from bone marrow.[64] Similarly, experiments have shown that osteoclasts can only be cultured if the surrounding micro-environment is favourable, and then only if direct cell-cell contact with living osteoblasts occurs.[65]

Physiological osteoblasts produce factors which stimulate osteoclastogenesis under the influence of osteotropic hormones and cytokines. These factors are expressed on the plasma membrane of the osteoblastic stromal cells. Direct cell-cell contact then mediates differentiation of the osteoclast precursors.[5, 65, 70] Osteoblastic stromal cells produce M-CSF which facilitates maturation of osteoclast progenitor cells into osteoclasts. Similarly, the production of Interleukin-1, 6, 8 and 18, together with tumour necrosis factor (TNF) and parathyroid hormone receptor peptide (PTHrP) by osteoblasts, stimulates osteoclastogenesis.[65] A crucial initiator produced by osteoblastic stromal cells has recently been identified as the receptor activator of NF-Kß known as receptor activator of nuclear kappa ligand (RANKL). RANKL is a type-II transmembrane protein belonging to the TNF superfamily. Individuals with an absolute lack of RANKL expression are completely devoid of osteoclasts due to the absence of osteoclastogenesis. These individuals develop severe forms of osteopetrosis. Similarly, knockout mice lacking RANKL protein do not have osteoclasts with subsequent development of severe osteopetrosis as a result of defective bone resorption.[64] RANKL has been shown to directly stimulate the process of osteoclastogenesis even in the absence of cell-cell contact. Peripheral blood monocytes exposed to RANKL, are thus able to differentiate into osteoclasts without any osteoblast participation.[5, 71]

RANKL binds to RANK receptors located on pre-osteoclast cells. OPG, a second member of the TNF superfamily, has been identified as an inhibitor of the osteoclastogenesis process. OPG acts as a decoy receptor for RANKL to which it competitively binds. In so doing, the functions of RANKL in the osteoclastogenesis process are neutralised.[71]

Following the successful binding of RANKL to RANK, the pre-osteoclast cells are converted into TRAP positive mononuclear osteoclasts. It is at this stage, that the expression of several important transcription factors, including Mitf and Tfe3, facilitate fusion of the mononuclear osteoclasts to form mature multinucleated osteoclasts.[5]

2.3.4. Terminal differentiation of osteoclasts

Mononuclear TRAP-positive osteoclastic stromal cells undergo a process of fusion, resulting in the formation of large multinucleated giant cells. The exact mechanism of fusion is not completely understood. The round mononuclear stromal cells are thought to be the precursors of the MNGCs.[5] The prominent vascularity typical of the giant cell lesions is potentially a source of peripheral blood monocytes which are drawn to the site of involvement by the release of chemoattractant substances. Monocyte chemoattractant protein-1 (MCP-1) has been detected in GCT of bone. The expression of MCP-1 assists with monocyte recruitment.[72] In summary, osteoclast differentiation can only occur within an appropriate micro-environment, conducive to this process. The terminal differentiation phase is facilitated by cytokines and growth factors produced by the osteoblast-like spindled mononuclear stromal cells.

Immediately prior to the fusion process, the mononuclear osteoclasts increase the expression of Na^+/K^+ ATPase, H^+ATPase, calcitonin receptors and TRAP.[72] The actual physical fusion of the progenitor cells is mediated by several cell surface proteins including MFR, CD44 and CD47.

The presence of a bony surface or alternatively a focus of chronic inflammation in which optimal cell-cell contact is allowed, enhances the fusion of the osteoclasts and their mononuclear precursors. Local growth factors produced by the osteoblast-like spindled cells such as IL-1, M-CSF, interferon-γ and 1,25α $(OH)_2 D_{3,}$ promote the terminal differential process.[72]

Mitf expression at this time is deemed crucial, not only for the terminal differentiation process, but also for the actual fusion process. Experimental rat models showing a complete lack of Mitf go on to develop severe osteopetrosis. Osteoclasts in these experimental animals have multiple morphological defects including the lack of a ruffled membrane, decreased multinucleation and diminished bone resorptive ability.[8] Mitf expression is thus essential in both the actual fusion/multinucleation process as well as for the terminal differentiation and functional ability of osteoclasts. The presence of RANKL within these lesions stimulates expression of Mitf. Mitf is thus regarded as an essential component of the last phase of osteoclastogenesis.[8]

Figure 9: *Diagrammatic summary of the chain of events occurring in osteoclastogenesis. A stem cell differentiates into a mature active osteoclast in a process requiring the presence of multiple functionally important enzymes and growth factors. Adapted from Hershey CL, Fisher DE. Bone 2004; 34: 689-696.*

2.4 Mitf and Tfe3 expression in giant cell lesions of the jaws

2.4.1 Microphthalmia-associated transcription factor (Mitf)

Mitf is a basic helix-loop-helix/Leucine zipper (b-HLH ZIP) transcription factor which belongs to the Mitf-Tfe family. This family also includes Tfec, Tfeb and Tfe3.[11] The expression of Mitf is essential for the terminal differentiation of osteoclasts, melanocytes, mast cells and natural killer cells.[8, 9] Mutations of the microphthalmia gene result in a multitude of defects including the autosomal dominant conditions Type 2a Waardenburg syndrome and Tietz syndrome. Sensorineural deafness and multiple hypopigmentation defects are common features.[52, 53] Experimental mouse models with mutant Mitf alleles have ocular involvement manifested by smaller sized eyes, a lack of coat colour, deafness due to the absence of pigment cells in the inner ear and mast cell defects. Osteopetrosis is an occasional feature.[8, 10, 72, 73] To date, twenty mutant alleles, classified as either semi-dominant or recessive, have been described. Pigmentation defects occur in all known mutations whilst only two mutant alleles result in osteopetrosis. Semi-dominant mutations produce pigment defects in heterozygous mice while the recessive genotype produces a phenotype in homozygotes only.[10] The mutant alleles associated with osteopetrosis are semi-dominant.[8]

Mitf and its family members Tfec, Tfeb and Tfe3 are functionally similar. These proteins are characterised by the presence of a basic domain followed by an HLH-ZIP component. Target gene binding occurs through the basic domain during the process of transcription. The HLH-ZIP component mediates the dimerisation of these factors. Mitf is capable of homodimerisation with itself as well as heterodimerisation with any of the related b-HLH-ZIP proteins. The dimerisation process involves the coupling of two of these factors which facilitates positioning of

the basic domain within the major groove of a DNA molecule. Mitf functions by binding the DNA consensus sequence CA(C/T) (G/A) TG.[8, 11]

Mutations may occur either within the basic domain region or within the HLH-ZIP component. Basic domain defects prevent DNA binding whilst the unaffected HLH-ZIP component continues to form homo- and heterodimers with related b-HLH-ZIP proteins. Osteopetrosis-associated mutations are the result of defects within the basic domain region of the Mitf gene. DNA binding therefore does not occur, however, the HLH-ZIP component continuously dimerises resulting in sequestration of related transcription factors within non-binding complexes giving rise to an osteopetrotic phenotype. Recessive inheritance of Mitf HLH-ZIP mutations readily causes pigmentation defects but not osteopetrosis. This observation led to the identification of related HLH-ZIP factors which compensate for the lack of Mitf.[8, 10, 74]

A Norwegian rat species with a spontaneous mutation in the Mitf gene and an absolute lack of Mitf at birth, has been the focus of several studies.[73, 75] Affected rats present with smaller sized eyes, a lack of body and fur pigmentation, incisor tooth defects and osteopetrosis. The white coat colour and small eyes resulted in the designation *microphthalmia blanc (mib).* The mib mutation is expressed in homozygotes (mib/mib) and is an autosomal recessive trait. The skeletal manifestations of osteopetrosis are severe at birth with decreased numbers of poorly functional osteoclasts. Histological examination of skeletal sections confirms a marked reduction in the size of bone marrow cavities which contain few haematopoietic elements. There are no features of physiological bone remodelling. Examination of other organs shows marked extramedullary haematopoiesis. The most peculiar observation is spontaneous resolution of the osteopetrosis by the fourth postnatal week associated with a marked improvement in the osteoclast number and function and decreased skeletal sclerosis. Genetic analysis performed at

this time, shows persistence of the Mitf mutation with continued Mitf deficiency.[8, 73] It was thus assumed that an additional transcription factor be involved in the osteoclastogenesis process, functioning with Mitf as well as compensating for it in cases of complete deficiency.[75] After exclusion of Tfeb and Tfec, sufficient evidence has identified Tfe3 as the important Mitf dimerisation partner, which accounts for the phenotypic compensation.[10]

2.4.2 Transcription factor E-3 (Tfe3)

Initial reports of a transcription factor binding at μE3 site of the immunoglobulin heavy chain (IgH) enhancer, in the transcriptional activation and activity of B lymphocytes led to its designation "Transcription factor E3". Tfe3 together with Tfeb, Tfec and Mitf, constitute the Mitf-Tfe family of b-HLH-ZIP proteins. Subsequent examination of the HLH-ZIP component of Tfe3 confirms its ability to mediate interactions with other proteins, the resultant homodimer and heterodimer interactions facilitating DNA binding.[73] Tfeb, Tfec and Tfe3 are all able to homodimerise with themselves as well as forming heterodimers with Mitf and each other.

Tfe3 is expressed abundantly in osteoclasts with no detectable expression in osteoblasts. It readily forms co-immunoprecipitates with Mitf. High levels of Tfe3 protein have consistently been detected within the Mitf immunoprecipitates of osteoclasts constituting as much as ten percent of the total Tfe3 protein expressed. The Mitf-Tfe3 heterodimerisation process therefore signifies the role of Tfe3 in osteoclast function and bone resorption.[8] Tfe3 and Mitf are expressed at similar levels in osteoclasts with low tissue-specific threshold levels suggesting the two proteins are able to replace each other.[72]

The spontaneous resolution of osteopetrosis in mib-rats led to the recognition of an additional transcription factor which compensates for Mitf deficiency. Mitf's ability to heterodimerise with

Tfec, Tfeb and Tfe3 resulted in several genetic studies to determine which of these three was responsible for the improved osteopetrotic phenotype.

Tfeb is essential in placental vascularisation with Tfeb gene mutations resulting in embryonic death. The role and significance of Tfeb in osteoclast, mast cell and melanocyte function is therefore difficult to establish as homozygous mutations are incompatible with life.[72]

Experimental generation of homozygous Tfec and Tfe3 mutant mice by means of gene knockout results in a normal phenotype. Mutations of either one of these two factors alone, does not appear to be critical in osteoclast development. In addition, heterozygous Tfec mutations, alone or in combination with other defective family members does not result in exacerbated osteopetrosis. Tfec is deemed non-essential in mammalian development.[10, 72]

Mitf and Tfe3 mutations occuring alone are also of little clinical consequence. Experimental double mutant knockouts for both Mitf and Tfe3, however, show features of severe osteopetrosis with complete obliteration of all marrow cavities and delayed tooth eruption. Double Mitf/Tfe3 mutant mice die within three weeks.[72] Tfe3 is thus implicated as the additional transcription factor essential for osteoclast differentiation and activation. The expression of Tfe3 is considered the most likely cause of the improved osteoclast phenotype in the mib-rat.[10]

2.4.3 The role of Mitf and Tfe3 expression in osteoclasts

The hereditary disorder of osteopetrosis is characterised by increased bone density/osteosclerosis. The disorder arises in the setting of defective physiological bone remodelling secondary to dysfunctional osteoclast activity.[12] Mitf is an essential downstream factor in osteoclastogenesis with deficiencies known to cause osteopetrosis.[9] Tfe3 has been shown to function synergistically with Mitf in the terminal differentiation of osteoclasts.[72]

Expression of both Mitf and Tfe3 is activated by several signalling pathways. M-CSF and RANKL activate Mitf and Tfe3 expression by means of phosphorylation. M-CSF specifically phosphorylates Mitf at serine 73 which contributes to osteoclast fusion and maturation. RANKL triggers the phosphorylation of Mitf on serine 307, which is essential for the activation of the TRAP promoter.[14] Mitf and Tfe3 in turn, regulate the expression of several enzymes which are critical for osteoclast function. TRAP and cathepsin K are two of the important transcriptional targets of both Mitf and Tfe3.[76]

Osteoclast-associated receptor (OSCAR) is a member of the leukocyte receptor complex family. It is only expressed by osteoclasts where it acts as an important factor in osteoclast differentiation by mediating cell-cell interactions with osteoblasts. The expression of OSCAR is controlled by Mitf.[77] In addition; Mitf regulates the expression of bcl-2 in osteoclasts and melanocytes. Bcl-2 is essential for endochondral bone formation with knockout mice showing features of osteopetrosis.[78] E-cadherin, a cell adhesion molecule, is an additional transcription target of Mitf in osteoclasts. The decreased expression of E-cadherin in Mitf deficiency may thus underlie the defective osteoclast fusion process.[79] The heterodimerisation of Mitf and Tfe3 results in significantly increased target gene activation, most notably of TRAP, more so than either factor working alone.[12]

The genes regulated by Mitf and Tfe3 are central to the bone resorbing ability of osteoclasts and as such, are located downstream in osteoclast differentiation. Analysis of Tfe3 and Mitf expression in the giant cell lesions of the jaws would therefore be useful in determining the exact nature of both the stromal and giant cells as well as for establishing the cellular interactions that exist between these cell types

CHAPTER 3

3.0 AIMS AND OBJECTIVES

3.1 Aim

To analyse the expression of immunohistochemical markers of osteoclast differentiation in a subset of giant cell-containing lesions of the jaws and thereby enhance our understanding of the biological nature of both the mononuclear stromal cells and multinucleated giant cells within these lesions.

3.2 Specific objectives

3.2.1 To examine the expression of the transcription factors Mitf and Tfe3 which are essential in osteoclastogenesis, in CGCG, PGCG, cherubism and ABC.

3.2.2 To compare the expression of Mitf and Tfe3 within the mononuclear stromal cells and multinucleated giant cells within CGCG, PGCG, cherubism and ABC.

3.2.3 To compare Mitf and Tfe3 expression within the mononuclear stromal cells and multinucleated giant cells between CGCG, PGCG, cherubism and ABC.

CHAPTER 4

4.0 MATERIALS AND METHODS

4.1 Biopsy material

Surgical excision specimens previously diagnosed as CGCG (n=10), PGCG (n=10), cherubism (n=5) and ABC (n=5), were retrieved from the archives of the Division of Oral Pathology, University of the Witwatersrand. The routine haematoxylin and eosin stained sections were reviewed in order to confirm the diagnosis in each case. The paraffin-embedded tissue sections were then cut and stained immunohistochemically. The tissue sections had originally been fixed in 10% neutral buffered formalin for a period of 18-48 hours. Control tissue in the form of malignant melanoma and alveolar soft part sarcoma were used for Mitf and Tfe3 respectively.

4.2 Immunohistochemistry

Immunohistochemistry was performed on deparaffinised 4µm sections using the following monoclonal antibodies:

- Mitf (Dako, Glostrup Denmark)
- Tfe3 (Santa Cruz Biotechnology, Inc. CA)

4µm sections of tissue were cut onto slides coated with 3-aminopropyltriethoxysilane (APES). The avidin-biotin complex method of immunohistochemical staining was used to stain these sections. Negative control sections were processed in the same way as for the experimental sections. Instead of the use of primary antibody in these cases, normal serum was used. Tissue derived from bony lesions was subjected to decalcification prior to embedding. In order to

determine the optimal working concentrations for the antibodies used in this study, a dilution gradient (1:25, 1:50, 1:100, and 1:200) was tested on preliminary tissue sections. The decalcification process hampers antigen retrieval, interfering with immunohistochemical staining. These initial tests showed the antibody dilutions of 1:100 for undecalcified tissue sections and 1:50 for decalcified sections to be most effective.

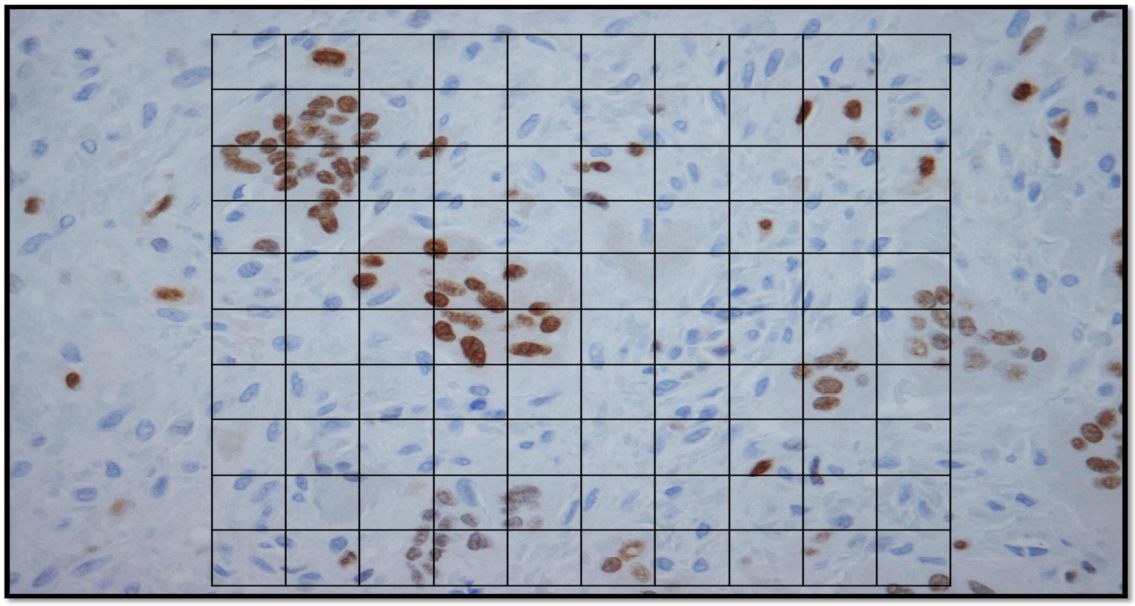
The deparaffinised sections (xylene, 2 x 5 minutes) were microwaved in a 0,01M citrate buffer at a pH of 6, 0 for 2 x 5 minutes (800W, medium power). When cool (after 20 minutes), the sections were immersed in 0,3% methanol hydrogen peroxide in buffer for thirty minutes to abolish endogenous peroxidase activity after which they were washed and then incubated with normal goat serum (1:10, 20 minutes). They were then treated with primary antibody serum at a dilution of 1:100 for routinely fixed soft tissues and 1:50 for decalcified tissues at room temperature for one hour. After washing with phosphate-buffered saline (PBS, pH 7, 6), the sections were treated with non-isoform specific antibodies for both Mitf and Tfe3 respectively with Envision plus (Dako) on a DAKO autostainer. Bound peroxidase was visualized by a 3, 3'-diaminobenzidine hydrochloride solution (DAB, Sigma) for five minutes resulting in a brown reaction product. Sections were counterstained with Meyer's haematoxylin (2 minutes) and mounted. All reagent dilutions and washings were performed in 0,01M PBS.

4.3 Immunohistochemical evaluation

Weak pale to strong dark nuclear staining of both the round mononuclear stromal cells and MNGCs was interpreted as positive for Mitf and Tfe3 in all four lesions included in this study. The most representative areas were located by means of scanning under low power magnification with a x4 objective. Trial counts were first performed in order to standardise the

methodology for optimal evaluation. A modified stereological method for determining the percentage of positive stromal cells and MNGCs for Mitf and Tfe3 respectively was used to confirm the final counts in the selected cases. This was achieved by using an eyepiece graticule containing 100 squares fitted to a Nikon microscope at x10 oculars and x40 objective in conjunction with a haematology DC counter (Nedtex Company). Ten of the most representative fields were selected from each tissue section (Fig. 10). In each field, the cells in 50 blocks of the grid were counted by using predetermined blocks located in alternate rows of the grid. Alternate rows were selected to simplify the counting process which may be hindered to some extent by overlapping nuclei or nuclei that lie in more than one block. This is most notable when counting giant cells which frequently occupy successive rows as demonstrated in figure ten. Nuclei touching the borders of the blocks were included in the count. The percentage labelling index for each of the two cell types was then calculated by dividing the number of positively stained lesional cells by the total number of lesional cells, multiplied by 100. The data obtained in this way was analysed statistically.

Figure 10: *The photomicrograph below diagrammatically illustrates the method used to count the number of positive stromal and MNGCs for the expression of Mitf and Tfe3. Ten of the most representative fields were selected from each section and examined by means of a counting grid containing 100 squares. Stromal and MNGCs showing positive nuclear staining were counted in 50 squares located in alternate rows of the grid in each of the selected fields.*



4.4 Observer variability

Intra-observer variability was tested by the prime examiner evaluating each of the fields twice with the resultant production of two sets of data labelled “observation 1” and “observation 2” respectively. Interobserver variability was similarly tested by a second examiner performing cell counts on selected cases using the exact same fields, independently. The set of data obtained from the second examiner was labelled “observation 3”. The two sets of data recorded by the prime examiner were statistically compared to each other for intra-observer variability whilst the first and second sets of matched data were compared to the third set recorded by the second examiner to assess inter-observer variability.

4.5 Statistical analysis

The raw data were entered into the Microsoft Excel 2010 programme. In addition, they were re-entered and tabulated as represented in Annexure A (see Appendix). The sets of data were then statistically analysed using the Statistical Package for Social Sciences (SPSS^{R}), version 19, statistical software programme.

The total number of positively stained stromal and giant cells counted in each case was divided by the total number of each cell type and then multiplied by 100 to obtain the mean labelling index. The mean labelling indices were used to calculate the means, standard deviation and range including minimum and maximum values, for both stromal cells and giant cells for Mitf and Tfe3 expression in PGCG, CGCG, cherubism and ABC respectively. Observations 1 and 2 were performed by the prime examiner whilst observation 3 was performed by a second examiner. The second examiner observed Mitf and Tfe3 expression in selected cases only, in order to assess interobserver variability.

The sets of data obtained by the prime observer (observations 1 and 2) were compared with each other to determine intra-observer variability. Matched data from the prime observer was compared with the data recorded by the second examiner (observation 3) in determining interobserver variability.

All sets of data were tested for normality of distribution by means of the Kolmogorov-Smirnov normality test. The null hypothesis for this test states that a variable is normally distributed. The hypothesis is rejected if the probability value is less than 5%.

Intra- and Interobserver variances for the mean labelling indices obtained for Mitf and Tfe3 in both the mononuclear stromal cells and MNGCs were analysed statistically by means of the

Wilcoxon matched pairs test as the data was deemed non-parametric (Table 1). The Spearman correlation test was then used to calculate rank correlation coefficients ("r") in order to assess how closely the results matched each other (Table 2). The results were represented graphically by means of scatterplots (Figs. 11-16).

The expression of Mitf and Tfe3 within the stromal and giant cells was compared within each of the four lesions included in the study by means of the Wilcoxon matched pairs test (Table 5). The set of data recorded by the prime examiner (observation 1) were used for this purpose.

The expression of Mitf and Tfe3 in both the stromal cells and giant cells was then compared across all four lesions (Table 6). The mean labelling indices obtained by the prime observer (observation 1) were compared in each case by using the Kruskal-Wallis test. The Kruskal-Wallis test is used statistically as a one-way analysis of variance by means of ranks, applied to non-parametric data. The test does not assume a normal population as is the case in the analogous one-way analysis of variance (ANOVA). It does, however, assume an identically shaped and scaled distribution for each group, except for any differences in medians.

4.6 Ethical considerations

The ethics code M080850 used for this research project adheres to international ethical norms for research. It provides blanket approval for the use of archival tissue block material, obtained from human subjects, and allocated to the Department of Anatomical Pathology, of which Oral Pathology is a Division, by the committee for Research on Human subjects (medical) of the University of the Witwatersrand (Annexure B).

CHAPTER 5

5.0 RESULTS

5.1 Intra- and interobserver variability

Intra-observer variability was assessed by comparing the mean labelling indices obtained by the prime examiner on two separate occasions (observations 1 and 2) in both stromal and giant cells for PGCG, GCGCG, cherubism and ABC respectively. For inter-observer variance, the mean labelling indices obtained by the prime examiner were similarly compared with those obtained by the second examiner (observation 3). The Wilcoxon matched pairs test for non-parametric data was applied to obtain the respective p-values (Table 1). The level of significance was set at 5%. The p-values in each case were greater than 0,05, indicating no statistically significant differences in the data obtained by the two examiners.

Correlation coefficients were computed using the Spearman correlation test for non-parametric data (Table 2). The correlation was consistently high for both intra- and interobserver comparisons. The degree of correlation is further depicted graphically by means of scatterplots (Figs. 11-16).

Table 1: *Intra- and interobserver comparison of Mitf and Tfe3 expression within all four lesions examined as calculated using the Wilcoxon matched pairs test for non-parametric data. T test values (parametric data) are displayed for comparison.*

Observations	Cell type	p-values						Number of observations
		Mitf		Tfe3		Mitf and Tfe3		
		T test	W test	T test	W test	T test	W test	
1 and 2	stromal	0,409	0,465	0,117	0,068	0,059	0,360	600
	giant	0,152	0,109	0,650	0,655	0,147	0,800	600
1 and 3	stromal	0,964	1,000	0,122	0,068	0,315	0,345	300
	giant	0,974	1,000	0,677	1,000	0,872	0,753	300
2 and 3	stromal	0.992	1,000	0,168	0,144	0,256	1,000	300
	giant	0.995	1,000	0.677	0,916	0,852	0,866	300

Table 2: *The Spearman correlation coefficient "r" is depicted for both intra-observer (observations 1 and 2) and interobserver (observations 1 with 3; and 2 with 3) comparisons of Mitf and Tfe3 expression in stromal and giant cells within all four giant cell lesions included in the study.*

Obser-vations	Cell type	Mitf		Tfe3		Mitf and Tfe3 combined	
		Correlation coefficient (r)	Number of observations	Correlation coefficient (r)	Number of observations	Correlation coefficient (r)	Number of observations
1 and 2	stromal	0,9899	300	0,9988	300	0,9981	600
	giant	0,98709	300	0.9998	300	0,9945	600
1 and 3	stromal	0,9167	150	0,9893	150	0,9858	300
	giant	0,8937	150	0,9560	150	0,9303	300
2 and 3	stromal	0,9233	150	0,9905	150	0,9872	300
	giant	0,8940	150	0,9561	150	0,9304	300

Figure 11: *Intra-observer variability. The scatterplot below graphically depicts the correlation of all observations noted by the prime examiner (observations 1 and 2) for Mitf and Tfe3 expression in the stromal cells of all four lesions included in the study.*

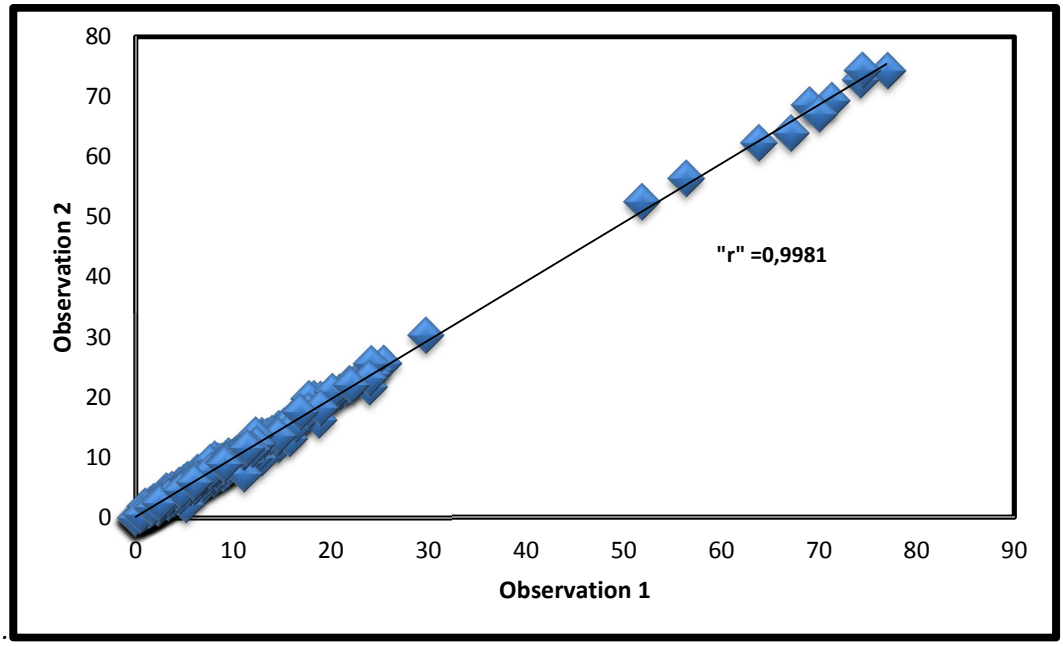


Figure 12: *Intra-observer variability. The scatterplot below graphically depicts the correlation of all observations made within the giant cells by the prime observer (observations 1 and 2) for Mitf and Tfe3 expression in all four lesions included in the study.*

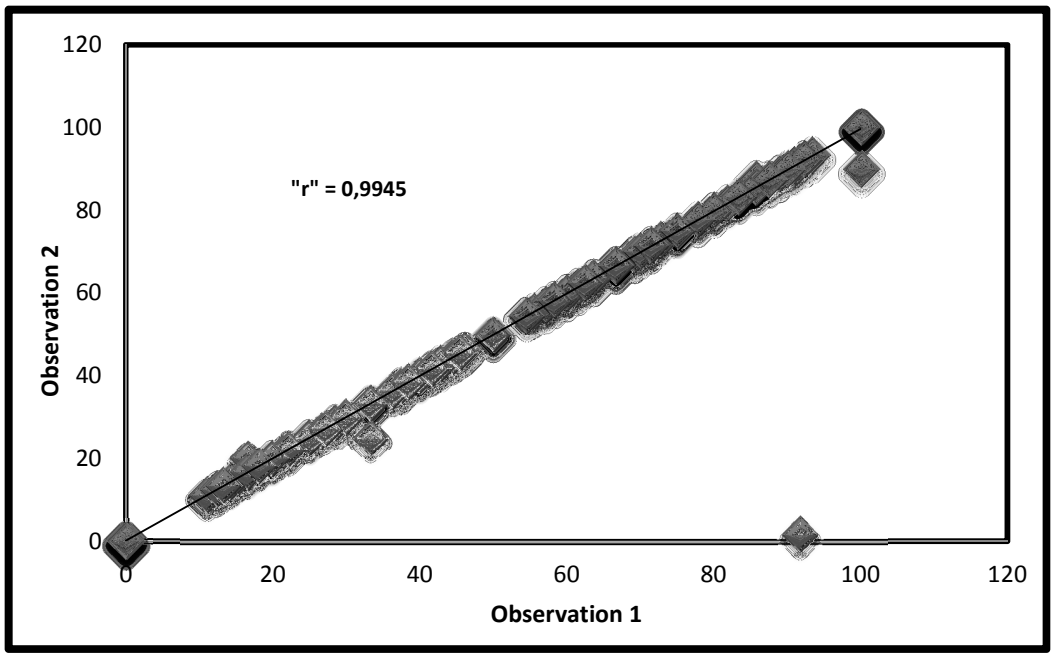


Figure 13: *Interobserver variability. The scatterplot below graphically depicts the correlation between the two independent examiners (observations 1 and 3) for Mitf and Tfe3 expression within the stromal cells of all four lesions included in the study.*

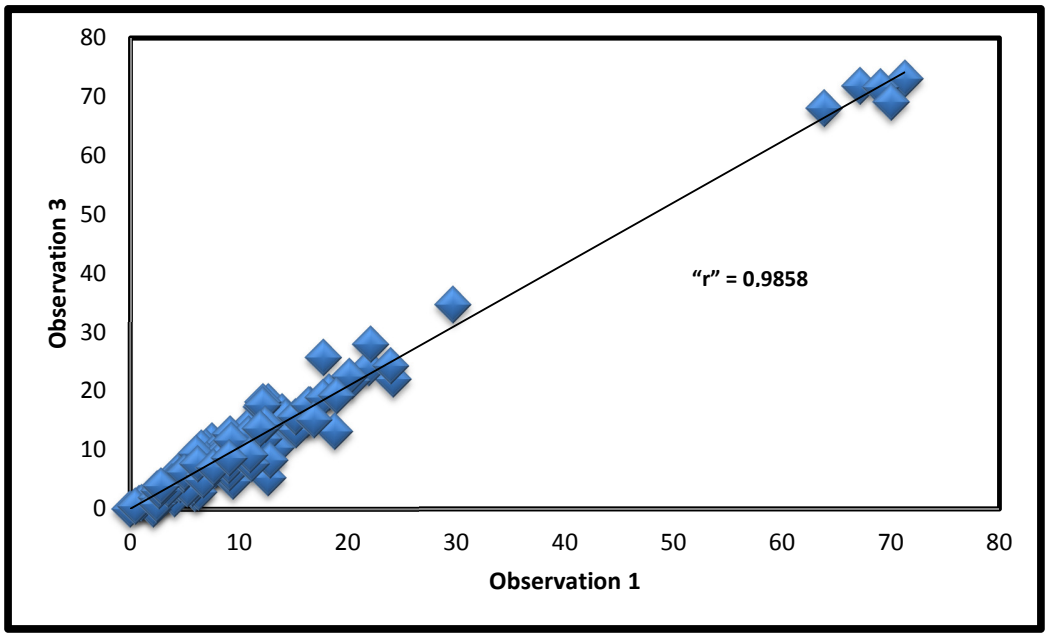


Figure 14: *Interobserver variability. The scatterplot below graphically depicts the correlation between the two independent examiners (observations 1 and 3) for Mitf and Tfe3 expression within the giant cells of all four lesions included in the study.*

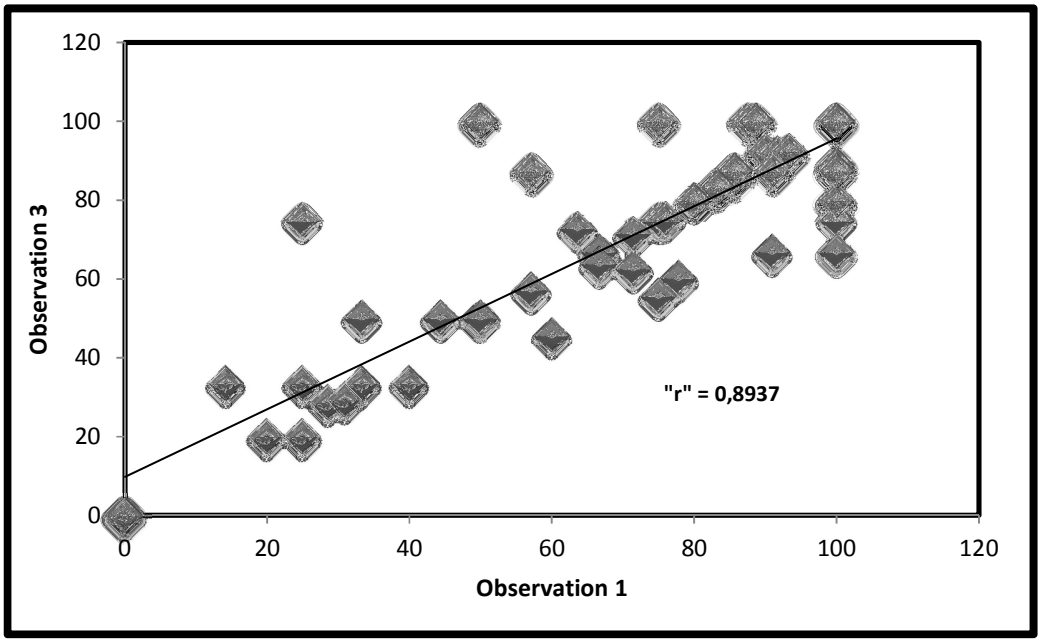


Figure 15: *Interobserver variability. The scatterplot below graphically depicts the correlation between the two independent examiners (observations 2 and 3) for Mitf and Tfe3 expression within the stromal cells of all four lesions included in the study.*

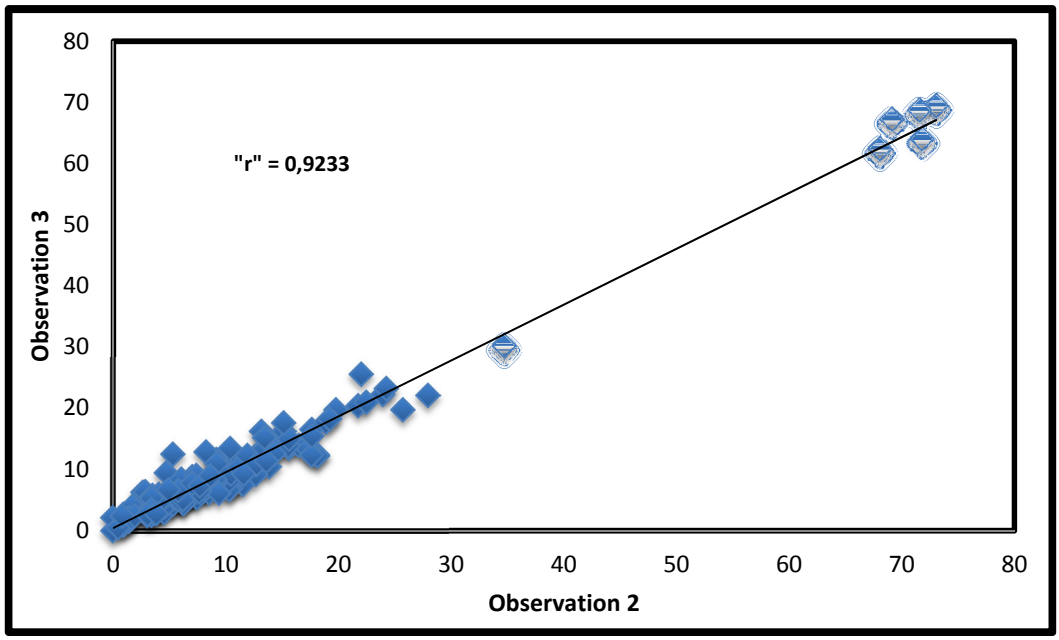
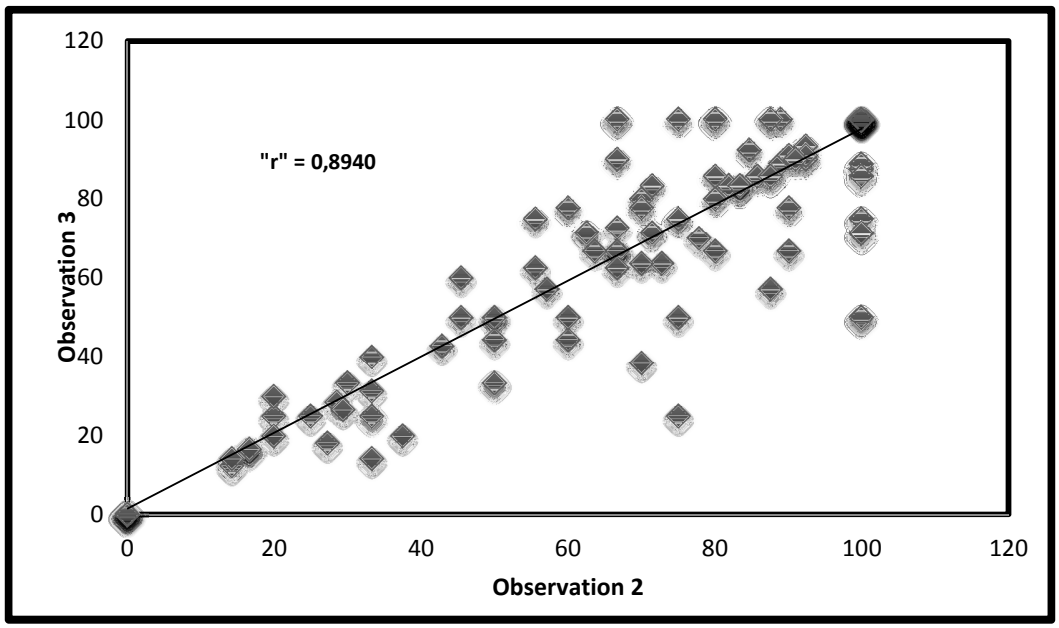


Figure 16: *Interobserver variability. The scatterplot below graphically depicts the correlation between the two independent examiners (observations 2 and 3) for Mitf and Tfe3 expression within giant cells of all four lesions included in the study.*



5.2 Mitf immunoreactivity

Mitf nuclear signalling was assessed in the stromal and MNGCs of PGCG, CGCG, cherubism and ABC (Figs.17-20). Positive nuclear staining was consistently higher within the MNGCs of all four lesions compared with stromal cell staining. The stromal cells exhibiting nuclear positivity were all plump with an ovoid to round nuclear morphology and constituted only a small percentage of the total number of stromal cells.

The mean, standard deviation and range for Mitf expression in the stromal and giant cells was obtained from the mean labelling indices recorded by the prime observer (observation 1) in PGCG, CGCG, cherubism and ABC. The results are summarised in Table 3.

For ease of comparison, the documented mean labelling indices for Mitf expression in both stromal and giant cells within PGCG, CGCG, cherubism and ABC have been depicted diagrammatically by means of a histogram in Figure 21. The results show consistently higher expression of Mitf within the MNGCs compared to stromal cells in each of the four giant cell lesions studied.

Figure 17: *Mitf expression in central giant cell granuloma. The multinucleated giant cells show diffuse positive nuclear signalling for Mitf whilst only occasional stromal cells are positive. (Mitf 4µm section; Original magnification: 400X).*

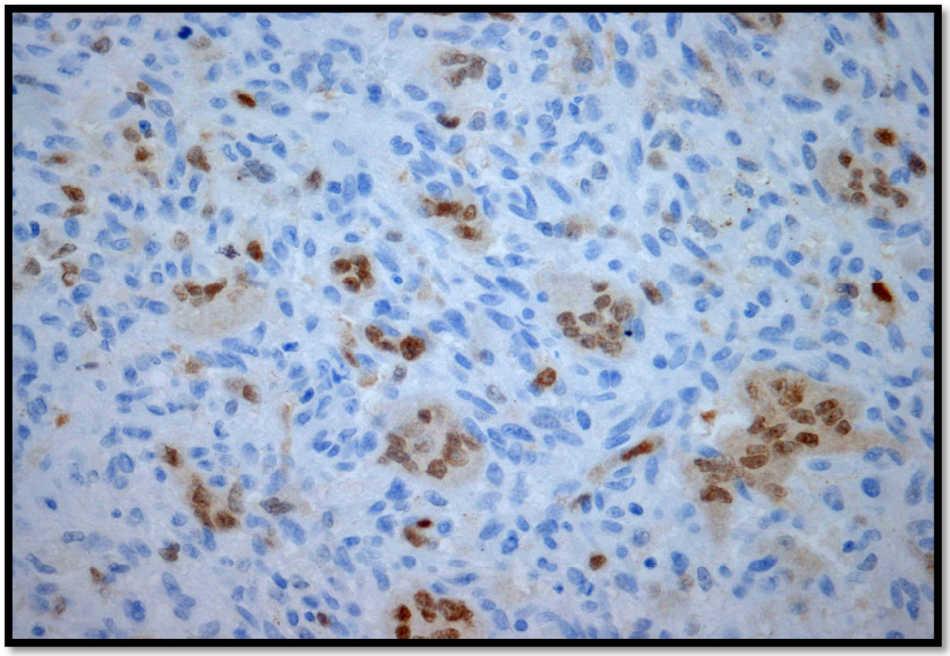


Figure 18: *Mitf expression in peripheral giant cell granuloma. Diffuse, strong nuclear immunoreactivity is observed within the giant cells compared to patchy positivity within fewer numbers of adjacent round mononuclear stromal cells. (Mitf 4µm section; Original magnification: 400X).*

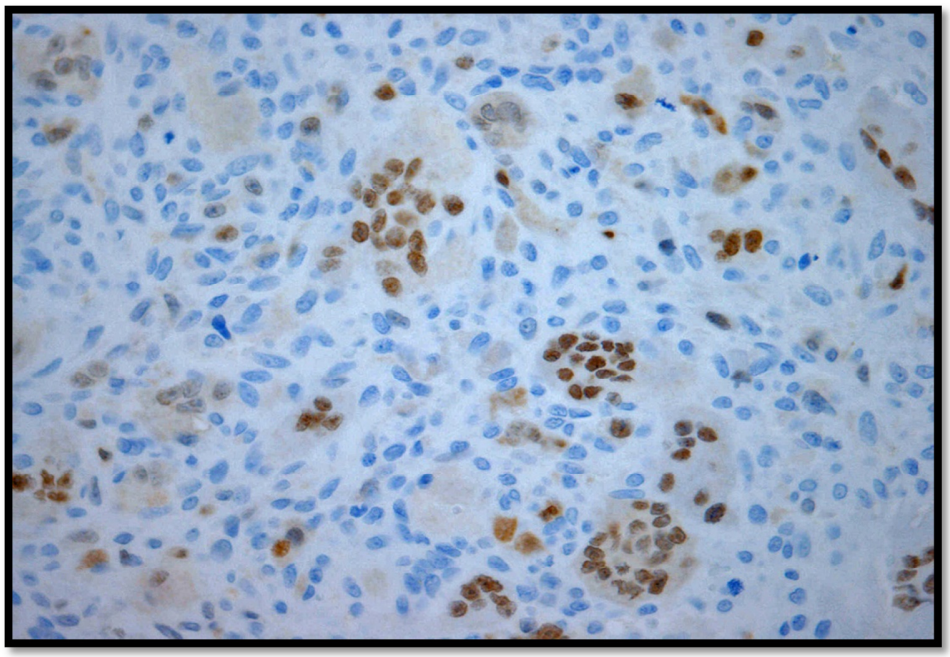


Figure 19: *Mitf expression in cherubism. Positive nuclear staining is noted within the nuclei of the giant cells with nuclear signalling in fewer of the adjacent mononuclear cells. (Mitf 4µm section; Original magnification: 400X).*

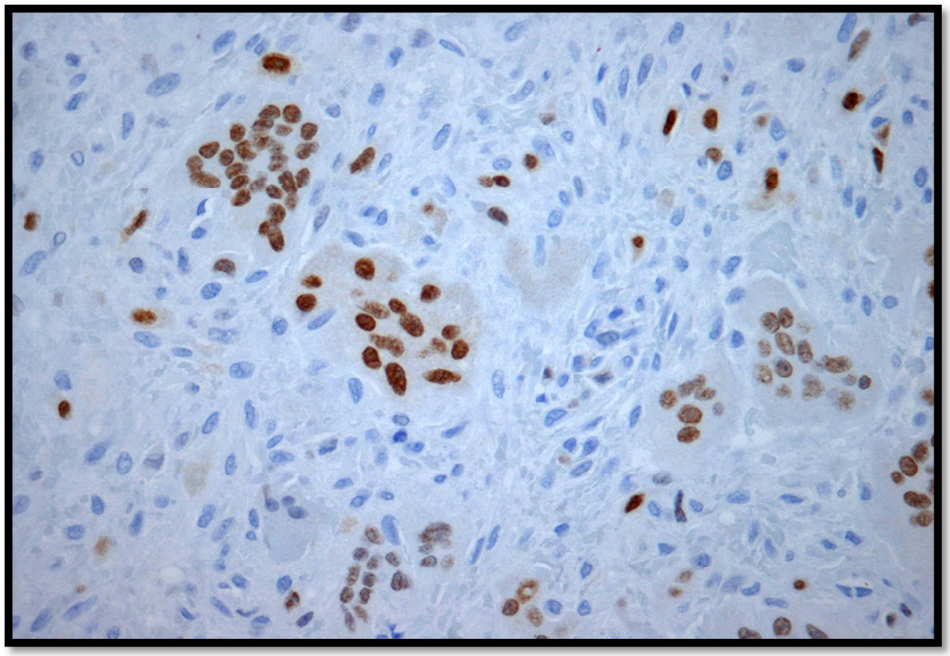


Figure 20: *Mitf expression in aneurysmal bone cyst. Positive nuclear staining is identified within the giant cell nuclei with fewer numbers of widely scattered positive stromal cells. (Mitf 4µm section; Original magnification: 400X).*

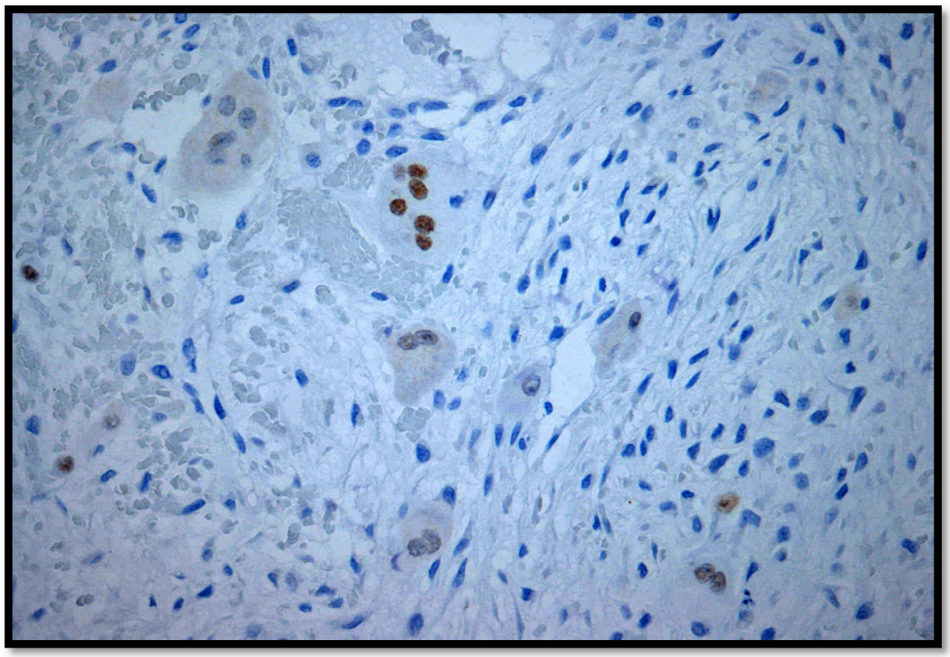
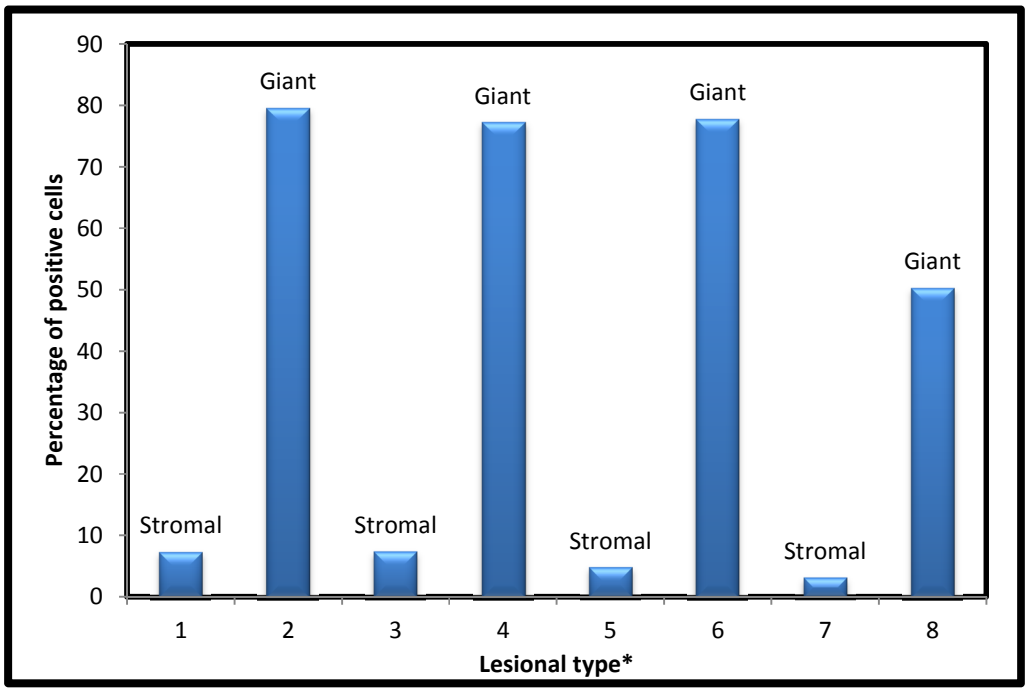


Table 3: *The table below summarises the descriptive statistical parameters for Mitf expression in both stromal and giant cells in all four lesions included in the study.*

Lesion	Cell Type	Mean	Median	Standard Deviation	Range	Minimum	Maximum
PGCG	Stromal	7,28	6,65	3,42	21	0	20,91
	Giant	79,61	88,75	27,80	100	0	100
CGCG	Stromal	7,34	6,51	4,25	19	0,5	18,75
	Giant	77,17	90	29,01	100	0	100
Cherubism	Stromal	4,73	4,14	3,14	14	0	14,41
	Giant	77,80	86,61	27,17	100	0	100
ABC	Stromal	3,08	2,69	1,77	8	0	7,69
	Giant	50,28	40	41,61	100	0	100

Figure 21: *Histogramatic representation of Mitf expression in the stromal and giant cells of all four lesions included in the study. The values were obtained from the mean labelling indices documented by the prime observer (observation 1).*



*Key to graph: 1&2 = PGCG, 3&4 = CGCG, 5&6 = cherubism, 7&8 = ABC

5.3 Tfe3 immunoreactivity

Nuclear staining for Tfe3 expression was assessed in the stromal and giant cells of PGCG, CGCG, cherubism and ABC (Figs. 22-25). The nuclear staining was once again notably higher within the giant cells of all four lesions compared with the stromal cell staining. Staining was, however, detected in fewer overall cells compared to Mitf. Mitf therefore appears to be preferentially expressed in all four giant cell lesions.

The mean, standard deviation and range for Tfe3 expression in stromal and giant cells of PGCG, CGCG, cherubism and ABC was obtained from the mean labelling indices recorded by the prime observer (observation 1). The results are summarised in Table 4.

For ease of comparison, the documented mean labelling indices for Tfe3 expression in both stromal and giant cells within PGCG, CGCG, cherubism and ABC are diagrammatically represented by means of a histogram in Figure 26. Tfe3 expression was consistently higher within the giant cells of all four lesions compared to the stromal cells. The results recapitulate those obtained for Mitf expression. The degree of staining, however, is much lower overall for Tfe3 expression as compared to Mitf expression.

Figure 22: *Tfe3 expression in central giant cell granuloma. Positive nuclear signalling is seen within the giant cells. Very focal, patchy positivity is seen within the nuclei of the adjacent stromal cells. (Tfe3 4µm section; Original magnification: 400X).*

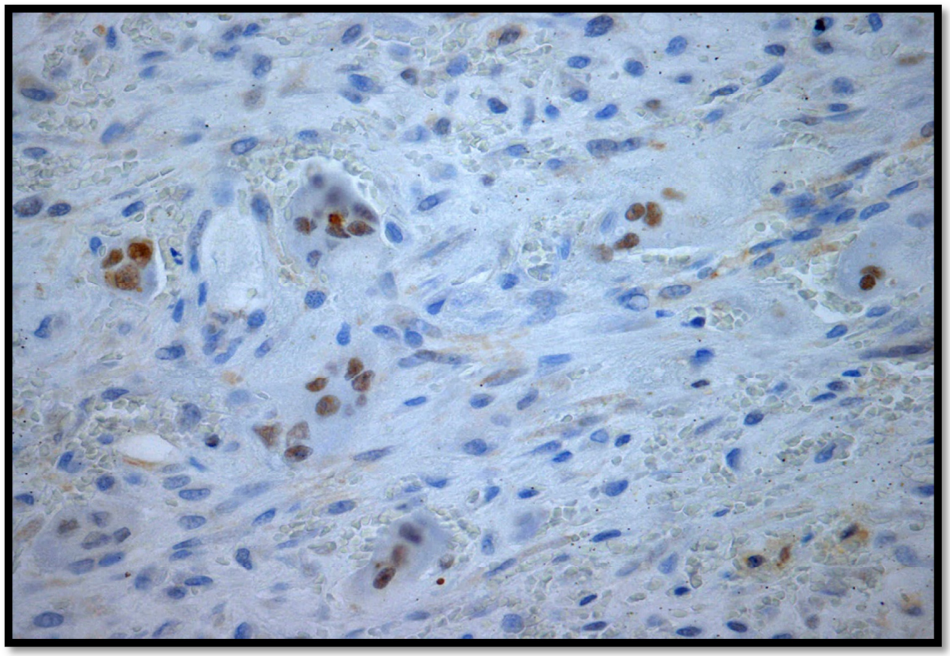


Figure 23: *Tfe3 expression in peripheral giant cell granuloma. Positive nuclear staining is observed within the giant cells with nuclear positivity in fewer of the adjacent round mononuclear stromal cells. (Tfe3 4µm section; Original magnification: 400X).*

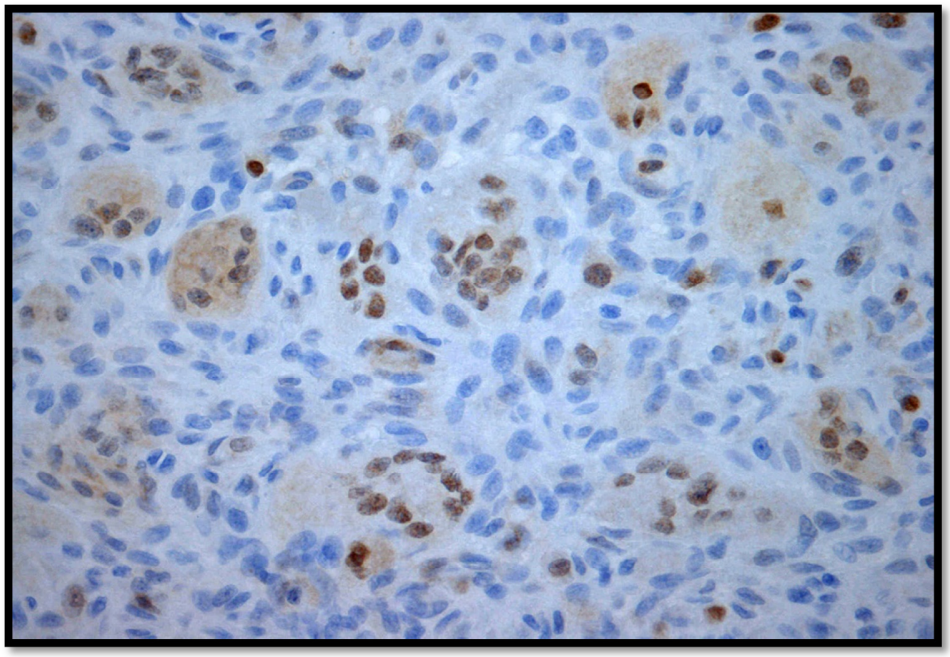


Figure 24: *Tfe3 expression in cherubism. Scattered multinucleated giant cells show strong nuclear positivity for Tfe3 with fewer adjacent positive stromal cells. (Tfe3 4µm section; Original magnification: 400X).*

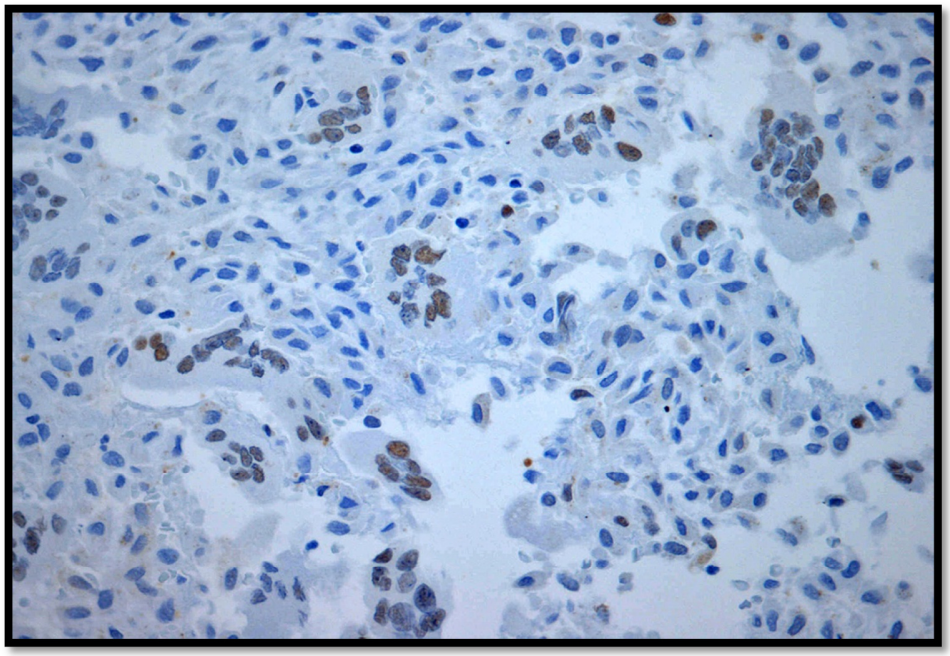


Figure 25: *Tfe3expression in aneurysmal bone cyst. Positive nuclear staining is observed within the cluster of giant cells in the centre with fewer positive stromal cells. (Tfe3 4µm section; Original magnification: 400x)*

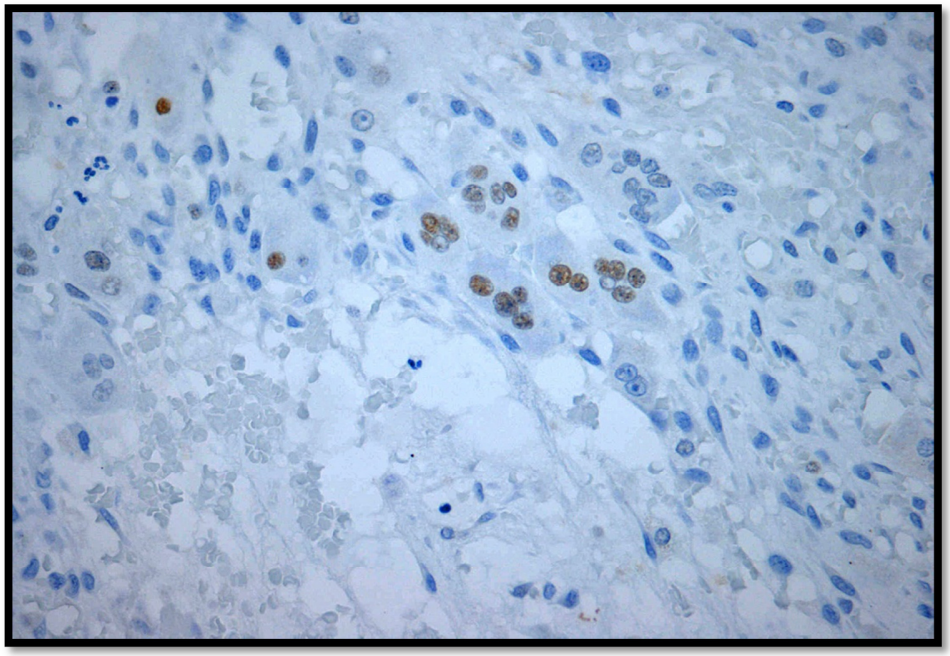
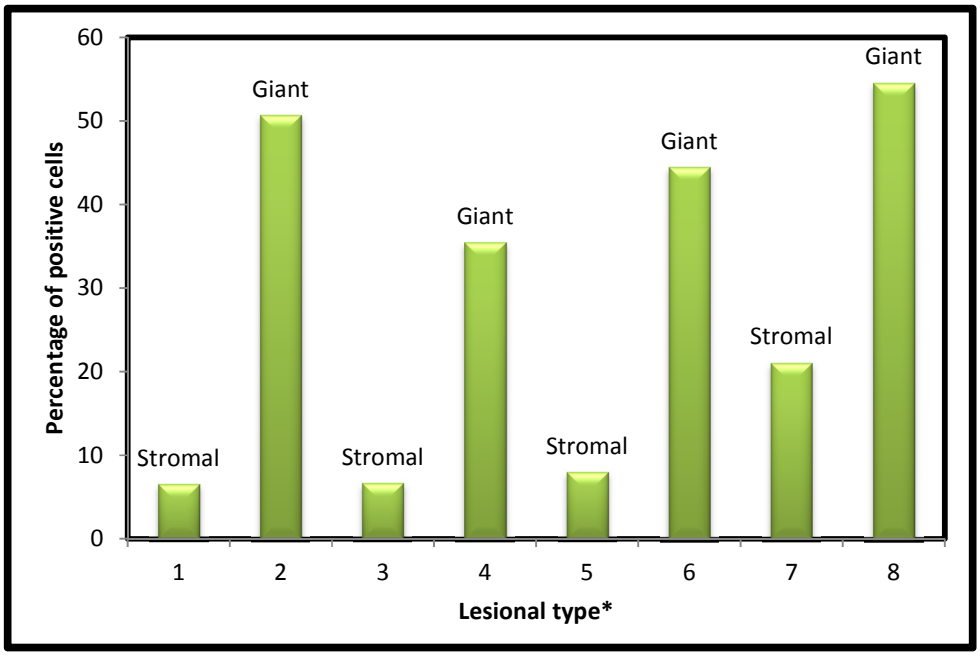


Table 4: *The table below summarises the statistical parameters for Tfe3 expression in both stromal and giant cells in all four lesions included in the study.*

Lesion	Cell Type	Mean	Median	Standard Deviation	Range	Minimum	Maximum
PGCG	Stromal	6,49	5,95	4,29	21,49	0,56	22,05
	Giant	50,64	50	32,16	100	0	100
CGCG	Stromal	6,64	5,18	5,66	29,69	0	29,69
	Giant	35,40	31,67	30,96	100	0	100
Cherubism	Stromal	7,90	6,98	5,34	20,12	0	20,12
	Giant	44,41	40	29,39	100	0	100
ABC	Stromal	21,03	9,79	24,65	75,36	1,55	76,92
	Giant	55,45	54,55	36,41	100	0	100

Figure 26: *Histogramatic representation of Tfe3 expression within the stromal and giant cells of all four lesions included in the study. The values were obtained from the mean labelling indices documented by the prime observer (observation 1).*



*Key to graph: 1& 2 = PGCG, 3&4 = CGCG, 5&6 = cherubism, 7&8 = ABC

5.4 Comparison of Mitf and Tfe3 expression between stromal and giant cells within each lesion

By using the mean labelling indices obtained by the prime examiner (observation 1), differences between Mitf and Tfe3expression were assessed in both the stromal and giant cells within PGCG, CGCG, cherubism and ABC in turn. This was achieved by means of the Wilcoxon paired sample test for non-parametric data (Table 5).

These results confirm differences in expression of Mitf and Tfe3 when the stromal cells and giant cells within each lesion are compared. In addition, significant differences in Mitf and Tfe3 expression are noted within each lesion. Mitf is consistently expressed at higher levels compared with Tfe3. Mitf, however, is consistently expressed at a higher level within both stromal cells and giant cells in each lesion.

5.5 Comparison of Mitf and Tfe3 expression within stromal and giant cells across the four lesions

The Kruskal-Wallis test, an analysis of variance statistical test for non-parametric data, was used to compare the expression of Mitf and Tfe3 by the stromal and giant cells between PCGC, CGCG, cherubism and ABC respectively (Table. 6). The tabulated results indicate the existence of statistically significant differences in the stromal cell and giant cell expression of both Mitf and Tfe3 across the four lesions included in the study. Overall expression of Mitf and Tfe3 was higher in PGCG, CGCG and cherubism compared with ABC with higher levels of Mitf being expressed in each case.

Table 5: *Comparison of Mitf and Tfe3 expression in stromal and giant cells by means of the Wilcoxon matched pair sample test within each individual lesion.*

Lesion	Cell Type	Transcription Factor	P-value
PGCG	Stromal	Mitf	0.064
		Tfe3	
	Giant	Mitf	**0.000**
		Tfe3	
CGCG	Stromal	Mitf	**0.004**
		Tfe3	
	Giant	Mitf	**0.000**
		Tfe3	
Cherubism	Stromal	Mitf	**0.003**
		Tfe3	
	Giant	Mitf	**0.000**
		Tfe3	
ABC	Stromal	Mitf	**0.000**
		Tfe3	
	Giant	Mitf	0.546
		Tfe3	

Table 6: *Comparison of Mitf and Tfe3 expression in stromal and giant cells by means of the Kruskal-Wallis test across all four lesions included in the study.*

Transcription Factor	Cell Type	Lesion	P-value
Mitf	Stromal	PGCG	**0.000**
		CGCG	
		Cherubism	
		ABC	
	Giant	PGCG	**0.001**
		CGCG	
		Cherubism	
		ABC	
Tfe3	Stromal	PGCG	**0.001**
		CGCG	
		Cherubism	
		ABC	
	Giant	PGCG	**0.001**
		CGCG	
		Cherubism	
		ABC	

CHAPTER 6

6.0 DISCUSSION

PGCG, CGCG, cherubism and ABC are reactive, non-neoplastic giant cell lesions of the jaws. The MNGCs within these lesions are osteoclastic in nature.[4-7, 9, 50-62] Aggressive CGCG, ABC and active lesions of cherubism may be associated with extensive osteolytic bone destruction. Surgery remains the mainstay of treatment, the extent of which is hugely debilitating, particularly in young patients in whom these lesions are most prevalent.

Mitf is an essential factor required for the terminal differentiation of osteoclasts. Tfe3 has been identified as an important cofactor in this process. Both transcription factors regulate the expression of several functionally important enzymes involved in osteoclastogenesis and bone resorption.[4, 12, 13] We have shown that Mitf and Tfe3 are expressed by both stromal cells and giant cells in PGCG, CGCG, cherubism and ABC. The expression of both transcription factors is significantly higher in the MNGCs compared to the adjacent stromal cells. This finding supports the concept of precursor stromal cell fusion, indicating that both transcription factors participate in the multinucleation process. It also reinforces the fact that the MNGCs have an osteoclast phenotype.

Our results strongly indicate that Mitf is preferentially expressed compared to Tfe3 within PGCG, CGCG and cherubism. Mitf is consistently expressed at a much higher level than Tfe3 in these lesions, thus indicating its role as the principal transcription factor in the terminal differentiation of the osteoclast-like giant cells. The role of Tfe3 is thus secondary to that of Mitf in osteoclastogenesis.

Mitf expression has previously been studied in a group of giant cell lesions.[9] Tfe3expression, however, has not been assessed or documented in any known giant cell lesions.

Seethala et al.[9] evaluated Mitf expression within the stromal and MNGCs in 89 giant cell lesions. These included GCT (n = 26), giant cell tumour of tendon sheath (n = 24), CGCG (n = 3), ABC (n = 11), chondroblastoma (n = 7), foreign body giant cell reactions (n = 10) and sarcoidosis (n = 8). In comparison to the current study, the lesions assessed previously were widespread and not site-specific. The three CGCGs documented occurred in the jaws while the ABCs were not restricted to any specific site. All three CGCGs and eight of the eleven ABCs showed Mitf immunoreactivity. The Mitf expression was predominantly located within the MNGCs and to a lesser extent in the adjacent round mononuclear stromal cells. These findings are supportive with regards to the concept of precursor stromal cell fusion to form the MNGCs. The results of the current study are similar in this regard. Furthermore, the previous study showed that lesions which contained no Mitf-positive MNGCs also lacked positively-stained stromal cells.

The methodology used in the study by Seethala et al.[9] was semi-quantitative. Mitf nuclear reactivity was evaluated by light microscopy and the percentage of positive nuclei was estimated for the mononuclear stromal cells and MNGCs separately. The exact method of estimation was not disclosed. The sample size of CGCGs was much smaller than in the current study whilst more ABCs were included. Studies describing Mitf expression in cherubism and PGCG were not found for comparison.

Liu et al.[5] employed a similar cell counting protocol to the one used in this study. They assessed the immunohistochemical expression of V-ATPase, CAII, cathepsin K, MMP-9 and CD68 in

PGCG (n = 6), CGCG (n = 34), cherubism (n = 7) and ABC (n = 6). Histochemical assessment of TRAP expression was also performed. Similar numbers of PGCG, cherubism and ABC were used as compared to the current study. There were, however, substantially more CGCGs compared to the current study. Enzyme activity and antibody expression were used as markers to confirm the osteoclast phenotype of the MNGCs. Positivity was noted largely within the giant cells with identification of very occasional positive round mononuclear stromal cells. This affirms the osteoclast-like nature of the giant cells whilst validating the stromal cell fusion hypothesis. The findings in the current study compare favourably with those noted by Liu B et al.[5] despite the use of different osteoclast markers. The cell counting technique employed by the same authors involved the random selection of five microscopic fields in each case. The percentage of positive stromal cells and MNGCs was assessed by counting at least one thousand of each cell type. The current study made use of ten randomly selected, representative microscopic fields in each of the thirty cases examined. We were able to count at least 865 stromal cells up to a maximum of 2385 depending on the cellularity and type of lesion. However, using twice as many microscopic fields compared to the previous study, we were only able to count between 23 and 115 MNGCs in each case. It therefore does not seem possible to assess 1000 MNGCs in as little as 5 microscopic fields. The stromal cells always outnumber the MNGCs in each HPF due to the sheer size of the MNGCs as well as their density and distribution. In addition, in some cherubism lesions, particularly those obtained at puberty or known to be in regression, a large proportion of the lesion consists of fibrous connective tissue with fewer stromal cells and scanty MNGCs.

Amaral FR et al[80] inadvertently documented the expression of the transcription factor NFATc1 in CGCG, PGCG and cherubism after initially investigating the presence of SH3BP2 mutations

in these lesions. SH3BP2 mutations are well known to occur in cherubism and have been described in a single case of CGCG but not in PGCG.[47] SH3BP2 activates the c1 isoform of NFAT which is recognised as a master transcription factor known to induce osteoclastogenesis. Increased expression of NFATc1 was documented in five cases of PGCG, five cases of CGCG and one case of cherubism by means of real time PCR and immunohistochemistry. The SH3BP2 gene is stimulated by RANKL expression which in turn regulates the formation of a complex containing variable proteins including the src family kinases, SYK, PLCγ2 and VAV. This protein complex increases intracellular calcium with resultant NFATc1 up-regulation, culminating in nuclear translocation of the molecule. The up-regulation of NFATc1 has both autocrine and paracrine effects and acts to regulate the expression of several important osteoclast-specific enzymes as well as mediating osteoclast fusion. Enzyme expression regulated by NFATc1 includes cathepsin K, TRAP, calcitonin receptor, αβ3 integrin and OSCAR.

NFATc1 therefore has similar functional activity to that of Mitf and Tfe3. NFATc1 expression is present predominantly within the giant cells in each of the three lesions described much in the same way that Mitf and Tfe3 expression is observed in the present study. The apparent restriction within the giant cells endorses the proposed osteoclast phenotype and confirms its role in the terminal differentiation process.

Torabinia N et al[60] compared expression of CD68 and TRAP within the stromal and giant cells of PGCG and CGCG. Both lesions showed strong positivity for both CD68 and TRAP with no detectable differences to account for the differences in osteolytic potential. The expression of CD68 and TRAP were again more notable within the giant cell component when compared to the stromal cells of both lesions in support of the osteoclastic giant cell nature and the presence

of the osteoclastogenesis process. The results are thus in agreement with those noted in the current study.

Matos FR et al[33] examined the expression of MMP-9, VEGF and von Willebrand factor (vWF) in twenty central and twenty peripheral giant cell lesions of the jaws. Immunohistochemical staining with vWF was used to assess the angiogenic index of these lesions. MMP-9 is identified as an essential molecule in angiogenesis as well as bone resorption. It is an enzyme that regulates the proteolysis of collagen. MMP-9 expression was noted to be strongly and diffusely positive within the MNGCs of both CGCG and PGCG and to a much lesser extent within the adjacent stromal cells. This reciprocates the results of several studies including the present. It functions to highlight the nature of the giant cells whilst promoting the theory of mononuclear stromal cell fusion.

VEGF is a potent chemoattractant pre-osteoclast agent. Its expression is documented within the stromal and giant cells of both central and peripheral giant cell lesions. A higher level of stromal VEGF is distinctly identified in CGCG compared with PGCG. High levels of expression are consistently noted even in those lesions which appear to be relatively avascular, thereby emphasising its osteoclastogenic role as opposed to its traditional role in angiogenesis. In addition, this study shows a negative correlation between VEGF expression and microvessel concentration, further upholding its role in osteoclastogenesis. The higher levels of VEGF in CGCG compared to PGCG may explain the notable differences in osteolytic potential between these two lesions. PGCG was noted to have a greater microvessel concentration which is thought to reflect its reactive nature. In the presence of persistent local irritation and surface ulceration, increased expression of pro-angiogenic factors and cytokines result in increased vascularity within these lesions.

In a follow-up study to further explain the behavioural differences between peripheral and central giant cell lesions, de Matos et al[60] examined the expression of TNF-α and TGF-β in CGCG and PGCG. Whilst positive expression for both was noted within the stromal and giant cells, higher levels of TNF-α were expressed in PGCG while higher levels TGF-β were evident in CGCG. TGF-β is known to promote the differentiation of osteoclasts and has been experimentally shown to induce osteoclastogenesis. Additional functions include the production of VEGF and induction of NFAT expression. The latter function requires co-ordinated collaboration of TNF-α with TGF-β. Osteoclastogenesis stimulated by TNF-α therefore depends on the production of TGF-β for NFAT regulation. The higher levels of TGF-β expression within the cells of CGCG therefore correlate with its greater propensity for bone destruction.

Papanicolaou P et al[81] recently compared expression of the osteoclastogenic cytokines TNF-α, Il-6 and Il-1β within the stromal and giant cells of forty CGCGs and forty PGCGs. TNF-α is a potent bone resorption inducing cytokine which also functions synergistically with Il-6 and Il-1β to bring about osteoclast differentiation and activation. Expression levels were notably higher within the giant cells compared to the stromal cells in both lesions further verifying the osteoclastic nature of the MNGCs. These results again show favourable similarity with those of several similar studies including the current one. Levels of TNF-α and Il-6 were higher in the stromal cells of CGCG compared to PGCG. Conversely, expression of Il-1β was greater in the stromal cells of PGCG compared the stromal cell expression in CGCG. The authors of this article speculate that TNF-α and Il-6 expression in CGCG may be responsible for enhancing the osteolysis of these lesions within a bony micro-environment. This may explain the superficial "cupping" erosion and surface demineralisation seen adjacent to PGCG lesions. The expression

of these osteoclastogenic molecules endorses the proposed osteoclast-like phenotype in agreement with the results obtained in the present study.

Houpis CH et al[82] examined the expression of parathyroid hormone-related peptide (PTHrP), parathyroid hormone-related receptor-1 (PTHR1), and the transcription regulator MSX1 in 20 cases of CGCG and 20 cases of PGCG. They showed that PTHrP and PTHR1 are expressed in the MNGCs and the round mononuclear stromal cells of both lesions. In addition, MSX1 was also expressed by both cell types. They concluded that immature osteoblastic cells were responsible for the activation of PTHR1-positive mature osteoblasts which produce RANKL. The RANKL then interacts with PTHrP/PTHR1-positive osteoclast precursors to induce osteoclastogenesis.

At least 9 isoforms of Mitf have been identified with expression appearing to be tissue-specific. The isoforms differ from each other in the region of the N-terminus which provides its own tissue specific properties. Each isoform is responsible for the recruitment of co-factors in the gene transcription process. The result of this recruitment process is formation of tissue specific ratios of the Mitf/Tfe transcription factor family members. Mitf A is an isoform of Mitf which appears to be ubiquitously expressed in both macrophages and osteoclasts. Lu SY et al[83] have recently demonstrated up-regulation of the isoform Mitf E in osteoclasts which is almost non-detectable in macrophages. In addition, they were able to show that RANKL-induced activation of Mitf is essential in osteoclastogenesis and that Mitf and Tfe3 may have distinct roles in this process. Some studies indicate that Mitf and Tfe3 are functionally redundant. This allows for compensation of one factor if the other is absent.[71] Lu SY et al[83] have, however, shown that Tfe3 has a limited capacity for Mitf compensation when there is an absolute deficiency thereof. The relationship between Mitf and Tfe3 may be more complex than initially anticipated. Tfe3 may

under normal circumstances, not be completely redundant in osteoclastogenesis and osteoclast function.

Investigation of Mitf and Tfe3 expression and activity in association with the biological behaviour of the giant cell lesions should be investigated. This may in turn be used predictively to separate those lesions which are potentially aggressive from the more innocuous lesions. A recent study has shown CD34 staining density to be a simple, useful histological tool for the separation of aggressive and non-aggressive lesions.[84] Assessment of Mitf and Tfe3 expression in relation to CD34 staining density would be may be a useful method of determining the biological behaviour of such lesions. CD34 staining density alone has been shown to be useful to adequately predict clinical outcome and behaviour in seventy percent of aggressive lesions and eighty-two percent of non-aggressive lesions. This has obvious therapeutic implications and may assist in determining the most effective treatment modality in some cases

Target therapy refers to the use of agents that specifically block the activation of lesional and neoplastic cells. This differs from conventional chemotherapy and radiotherapy which interfere with or inhibits only those cells that are rapidly dividing.[85] The identification of potential drug targets by molecular medicine is expected to allow for the development of targeted therapies with far greater efficacy and fewer side-effects. Mitf and Tfe3 expression as demonstrated in the present study, confirms the osteoclastic nature of the MNGCs in PGCG, CGCG, ABC and cherubism. This knowledge is useful for understanding the molecular processes involved in MNGC formation. Elucidation of the osteoclastogenic process within these lesions reveals potential therapeutic targets at almost every stage.

Mitf and Tfe3 both regulate the transcription of genes which are associated with bone resorption. Transcription targets shared by these two factors include collagenases of the MMP-family, cathepsin K, TRAP, CA II and V-ATPase.[60, 63-66] Of these enzymes, cathepsin K is first to act in the bone resorption process and has effective matrix degrading ability. It therefore represents an attractive therapeutic target in the prevention of osteolysis. Investigation into the activity of cathepsin K in this particular subset of giant cell lesions is therefore suggested.

Mitf is activated through phosphorylation by MAP kinase in response to c-kit signalling. This results in up-regulation of Mitf transcriptional activity. The expression of Mitf is modified by c-kit signalling. Ninety percent of osteoclast precursors have been shown to require c-kit signalling for differentiation. C-kit (CD117) activation may therefore be involved in the promotion of osteoclast formation.[86] Investigation of the role of c-kit dysregulation in these lesions thus warrants further investigation.

RANKL is crucial for osteoclastogenesis as seen in RANKL deficient individuals who develop severe osteopetrosis due to defective bone resorption.[64] RANKL may directly stimulate the process of osteoclastogenesis even in the absence of cell-cell contact. Peripheral blood monocytes exposed to RANKL are thus able to differentiate into osteoclasts without osteoblast participation.[5, 71] The inhibition of RANKL-mediated osteoclastogenesis is thus an attractive therapeutic target for further investigation within these lesions. Additional potential therapeutic targets in this regard include M-CSF, Il-1, 6, 8, 18, TNF and PTHrP. The inhibition of these factors may be of assistance in inhibiting RANKL-mediated osteoclastogenesis.

The current study shows reduced levels of expression for both Mitf and Tfe3 within the MNGCs of ABC compared with expression levels in the other giant cell lesions of the jaws. This finding

may in fact support the theory that ABC is a secondary, reactive phenomenon with lower osteolytic potential.

The cell counting technique used in this study produces a comprehensive, reproducible set of quantitative data, the advantage of which is greater detail and accuracy. Similar studies largely use qualitative or descriptive methods of assessing transcription factor expression. Semi-quantitative methods of estimating the percentage of positive cells in each case are also popular. However detailed our data may be, in reality, the counting protocol is a difficult, time consuming and labour intensive process. Ten randomly selected microscopic high power fields (HPFs) examined twice in quick succession by the prime observer, yielded cell counts of up to 4768 for the stromal cells and up to 230 for the giant cells in each case. This process had to be repeated for two antibodies in thirty cases with 60 HPFs ultimately being assessed. Selected fields in alternate cases were examined independently by the second observer immediately after examination by the first. Statistical analysis, however, confirms the reproducibility of the data. Correlation statistics confirms little intra- and interobserver variability.

An important point of consideration in studies of this kind is the existence of subjective interpretation. Our study protocol stated that for the evaluation of Mitf and Tfe3 immunoreactivity, a brown to clear-brown nuclear signal would be regarded as positive. In many of the cases examined, the nuclear signalling was extremely weak and inconsistent with some cells showing barely discernable pale nuclear stippling. This type of staining, while considered positive by one observer, may be completely disregarded by another. Many of the cases included in this study were obtained from archival material dating back some fifteen to twenty years. The antigen retrieval may be compromised in older cases resulting in weakly stained sections. In addition, most of the cases with the exception of PGCG were obtained from

mandibular or maxillary resection specimens. The decalcification process may interfere with antigen retrieval with suboptimal immunohistochemical staining. Furthermore, since many of the sections were initially processed over twenty years ago, there is no assurance of a standard operating procedure. The processing of the tissues will have been performed by several different people at different times.

Additional factors to consider include the differences in histomorphology between the four lesions. PGCG for example, is usually surfaced by oral mucosa which is most often traumatised and ulcerated. The result of this is significant inflammation within and adjacent to the lesional tissue. PGCG is typically a cellular lesion with an abundance of MNGCs. The presence of additional background inflammatory cells and enlarged endothelial cells hampers accurate assessment of the total number of stromal cells as well as of those which are positively stained. It is easy in such cases to inadvertently inflate the number of stromal cells in each field by including similar-appearing inflammatory cells, endothelial cells or fibroblasts. There is also often considerable overlap of stromal cell nuclei with each other and with background non-lesional cells which can result in significant subjective differences in interpretation with regards to the total number of cells.

Large expanses of lesional tissue are not always observed in ABC. The histological picture is often dominated by the presence of sinusoidal spaces filled with extravasated red blood cells with only a thin peripheral rim of lesional tissue showing the characteristic features of a giant cell lesion. These narrow strips of lesional tissue may account for the smaller overall number of stromal cells and giant cells observed in these lesions.

Lesions of cherubism may not always have the classical features expected of giant cell lesions, particularly specimens obtained from older patients in whom the lesions are already regressive in nature. These cases will show a predominance of stromal cells and fibrous connective tissue with fewer, smaller and relatively inactive MNGCs. These morphological differences are overcome to a certain extent by the selection of ten representative fields in each case.

At least 1000 stromal cells were counted in most cases, the larger the cell count, the more accurate and reproducible the results are. Larger cell counts result in fewer statistically significant differences. Giant cell assessment is much more challenging by comparison. This is due to the size of the giant cells as well as the discrepancies in distribution. Many HPFs contain far fewer giant cells resulting in smaller overall sample sizes. There is great variation in the size and shape of the MNGCs within lesions of the same type and between the different lesions examined. Occasional lesions show MNGCs with a large fractional surface area and up to a hundred nuclei whilst others are significantly smaller but have a greater giant cell density. In several sections, particularly those cases characterised by increased MNGC density, it is not uncommon to find areas within a HPF where three or more MNGCs overlap, creating difficulties in accurately recording cell totals. The individual cell membranes are obscured in these cases.

The giant cell lesions of the jaws are all characterised by an increased vascularity with an abundance of extravasational-type haemorrhage which is associated with widespread haemosiderin pigment deposition. Haemosiderin pigment is golden brown with a refractile quality facilitating distinction from similarly coloured pigments as well as the brown immunohistochemical reaction product. In the cellular, inflammatory-rich areas of tissue, the distinction is not always clear cut with resultant differential interpretation. This is most notable

when the haemosiderin pigment is superimposed on lesional cell nuclei. Background formalin pigment or melanin may further confound accurate analysis.

The thirty cases included in this study were not divided into aggressive and non-aggressive lesions either by use of clinical or radiographic features or by means of histological assessment as described previously and suggested in the literature. [21, 22]

Clinical features in keeping with an aggressive lesion include a history of rapid growth with marked swelling and facial distortion due to the large size of the lesion, site of involvement – particularly within the maxillary skeleton and posterior mandible, young age at presentation, features of pain and paraesthesia, perforation of the cortical plate and lesional recurrence following surgical intervention. Radiographic features suggestive of an aggressive lesion include large multilocular lesions, root resorption and displacement as well as cortical perforation.

Histological reports documenting brief clinical details for each patient were available for each of the cases in this study; however, most were not comprehensive enough to make such a distinction. Details such as the exact size of the lesion in each case or the exact site of involvement were not known for many of the cases. In addition, radiographs were not available for assessment. There is no known history of patient follow-up including documented lesional recurrence to allow for accurate correlation with the clinical behaviour.

As the cases in this study were not subclassified by any method, the degree of Mitf and Tfe3 expression cannot be correlated with the biological behaviour of the lesions. It would seem that increased expression of Mitf and Tfe3 would indicate increased osteoclastic differentiation which in turn would correlate with increased numbers of MNGCs, a feature known to be associated with aggressive behaviour.[21, 22] This may be an indication of the osteolytic potential of

the lesion. Mitf and Tfe3 expression could therefore also serve as an adjunct in the histological assessment of aggressiveness. This hypothesis of course does not hold true in PGCG where Mitf and Tfe3 expression is notable as is the number and density of MNGCs, yet lesions have little osteolytic potential.

CHAPTER 7

7.0 CONCLUSION

Mitf and Tfe3 are transcription factors involved in osteoclastogenesis. This study confirms the expression of both Mitf and Tfe3 in PGCG, CGCG, cherubism and ABC, a subset of giant cell-containing lesions occurring in the jaws. These results confirm the presence of osteoclast-like cells with osteolytic potential within these lesions.

Mitf and Tfe3 expression is consistently greater within the MNGCs compared to the stromal cells. Stromal cells expressing these two factors are round to ovoid in shape and are usually seen in the immediate vicinity of the adjacent giant cells. This supports the concept of giant cell formation through stromal fusion.

Mitf is expressed at similar levels in PGCG, CGCG and cherubism. It is expressed at a much lower level in ABC. Tfe3 expression is much lower when compared to Mitf in PGCG, CGCG and cherubism. Mitf is thus preferentially expressed within these lesions. Mitf and Tfe3 expression is comparable in ABC, with no evidence of preferential/differential expression.

Further evaluation of the downstream transcription targets of Mitf and Tfe3 in this subset of giant cell containing lesions is essential for molecular characterisation and the development of therapeutic drug targets. In addition to identification of downstream targets, molecular assessment of target gene promoters is required to identify any factors which activate or suppress promoter activity.[87] For this purpose, microarray analysis and gene expression profiling would be a useful tool in order to assess all upregulated genes with osteolytic potential.

Prospectively, next generation sequencing performed on fresh tissue samples may also be used to assess and compare the gene expression profiles of the various lesions. Once the lesional profiles have been observed, the identification of transcription factors which modulate gene expression and the factors that affect transcription factor up-regulation may be achieved.

Tfec has been identified as an additional member of the Mitf/Tfe transcription factor family to be expressed in osteoclasts and osteoclast-like MNGCs. It is traditionally considered to be repressive in its actions with Tfe3, inhibiting Tfe3-mediated transactivation. It lacks an acidic domain which in the case of Tfe3, confers the ability to activate transcription.[88] Little is known about its expression in normal cell lines and the relative tissue-specific ratios at which it occurs. Sufficient quantities of Tfec are suspected of repressing target gene activation. Its presence in a lesion such as PGCG may therefore be used to explain the lack of osteolytic activity of the MNGCs within this lesion. This merits further investigation and would also serve to clarify its role within these lesions.

The identification of molecular targets within the giant cell lesions of the jaws may ultimately result in development of non-surgical treatment options for more efficient and conservative disease management.

CHAPTER 8

8.0 APPENDIX

8.1 Annexure A - Raw data

8.1.1 Mitf Immunoreactivity

8.1.1.1 Mitf Immunoreactivity in PGCG

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	1	0	108	108	0.000	0	110	110	0.000				
TJL91/195	2	10	150	160	6.250	10	147	157	6.369	8	153	161	5.229
	3	1	122	123	0.813	1	123	124	0.806				
	4	7	156	163	4.294	7	156	163	4.294	8	142	150	5.333
	5	1	169	170	0.588	1	173	174	0.575				
	6	8	122	130	6.154	7	124	131	5.344	9	112	121	7.438
	7	4	145	149	2.685	5	141	146	3.425				
	8	7	101	108	6.481	7	99	106	6.604	8	108	116	6.897
	9	7	142	149	4.698	7	142	149	4.698				
	10	12	126	138	8.696	10	126	136	7.353	12	131	143	8.392
Total		**57**	**1341**	**1398**	**4.251**	**55**	**1341**	**1396**	**3.940**	**45**	**645**	**690**	**6.295**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	1	0	5	5	0.000	0	5	5	0.000				
TJL91/195	2	6	1	7	85.714	6	1	7	85.714	6	0	6	100.000
	3	0	8	8	0.000	0	8	8	0.000				
	4	6	2	8	75.000	6	2	8	75.000	5	4	9	55.556
	5	4	1	5	80.000	4	1	5	80.000				
	6	4	3	7	57.143	4	3	7	57.153	4	3	7	57.143
	7	3	3	6	50.000	3	3	6	50.000				
	8	3	0	3	100.000	3	0	3	100.000	3	1	4	75.000
	9	2	3	5	40.000	2	3	5	40.000				
	10	5	2	7	71.429	5	2	7	71.429	5	2	7	71.429
Total		**33**	**28**	**61**	**55.929**	**33**	**28**	**61**	**54.098**	**23**	**10**	**33**	**69.697**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	12	143	155	7.742	11	143	154	7.143				
TJL97/469	**2**	15	148	163	9.202	14	156	170	8.235				
	3	10	146	156	6.410	9	148	157	5.735				
	4	5	154	159	3.145	7	150	157	4.459				
	5	13	156	169	7.692	11	160	171	6.433				
	6	11	173	184	5.978	11	165	176	6.250				
	7	6	143	149	4.027	7	157	164	4.268				
	8	18	206	224	8.036	16	231	247	6.478				
	9	11	232	243	4.527	12	233	245	4.898				
	10	7	182	189	3.704	6	183	189	3.175				
Total		**108**	**1683**	**1791**	**6.030**	**104**	**1726**	**1830**	**5.683**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	12	0	12	100.000	12	0	12	100.000				
TJL97/469	**2**	6	0	6	100.000	6	0	6	100.000				
	3	7	0	7	100.000	7	0	7	100.000				
	4	6	0	6	100.000	6	0	6	100.000				
	5	5	0	5	100.000	5	0	5	100.000				
	6	4	0	4	100.000	4	0	4	100.000				
	7	0	0	0	0.000	0	0	0	0.000				
	8	5	0	5	100.000	5	0	5	100.000				
	9	1	2	3	33.333	1	2	3	33.333				
	10	4	0	4	100.000	4	0	4	100.000				
Total		**50**	**2**	**52**	**96.154**	**50**	**2**	**52**	**96.154**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	10	161	171	5.848	8	169	177	4.520				
TJL99/470	**2**	12	165	177	6.780	9	171	180	5.000				
	3	7	104	111	6.306	8	107	115	6.957				
	4	13	157	170	7.647	12	164	176	6.818				
	5	6	179	185	3.243	5	176	181	2.762				
	6	7	179	186	3.763	7	179	186	3.763				
	7	10	149	159	6.289	9	153	162	5.556				
	8	8	115	123	6.504	8	122	130	6.154				
	9	6	185	191	3.141	6	194	200	3.000				
	10	16	189	205	7.805	16	193	209	7.656				
Total		**95**	**1583**	**1678**	**5.662**	**88**	**1628**	**1716**	**5.128**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	6	1	7	85.714	6	1	7	85.714				
TJL99/470	**2**	3	2	5	60.000	3	2	5	60.000				
	3	3	0	3	100.000	3	0	3	100.000				
	4	7	0	7	100.000	7	0	7	100.000				
	5	6	1	7	85.714	6	1	7	85.714				
	6	6	0	6	100.000	6	0	6	100.000				
	7	4	0	4	100.000	4	0	4	100.000				
	8	0	2	2	0.000	0	2	2	0.000				
	9	11	0	11	100.000	11	0	11	100.000				
	10	6	2	8	75.000	6	2	8	75.000				
Total		**52**	**8**	**60**	**86.667**	**52**	**8**	**60**	**86.667**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	6	123	129	4.651	5	130	135	3.704				
TJL00/312	**2**	12	99	111	10.811	12	103	115	10.435	13	81	94	13.830
	3	1	124	125	0.800	1	118	119	0.840				
	4	8	126	134	5.970	9	121	130	6.923	10	114	124	8.065
	5	12	110	122	9.836	12	113	125	9.600				
	6	10	143	153	6.536	10	143	153	6.536	11	154	165	6.667
	7	12	105	117	10.256	12	102	114	10.526				
	8	20	128	148	13.514	18	130	148	12.162	18	125	143	12.587
	9	6	165	171	3.509	5	172	177	2.825				
	10	7	96	103	6.796	7	98	105	6.667	7	102	109	6.422
Total		**94**	**1219**	**1313**	**7.159**	**91**	**1230**	**1321**	**6.889**	**59**	**576**	**635**	**9.291**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	10	2	12	83.333	10	2	12	83.333				
TJL00/312	**2**	6	2	8	75.000	6	2	8	75.000	8	0	8	100.000
	3	6	4	10	60.000	6	4	10	60.000				
	4	10	1	11	90.909	10	1	11	90.909	8	4	12	66.667
	5	5	0	5	100.000	5	0	5	100.000				
	6	7	0	7	100.000	7	0	7	100.000	7	0	7	100.000
	7	7	0	7	100.000	7	0	7	100.000				
	8	4	0	4	100.000	4	0	4	100.000	7	0	7	100.000
	9	9	2	9	77.778	9	2	9	77.778				
	10	5	1	6	83.333	5	1	6	83.333	5	1	6	83.333
Total		**69**	**12**	**79**	**84.810**	**69**	**12**	**79**	**84.810**	**35**	**5**	**40**	**87.500**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	13	119	132	9.848	11	114	125	8.800				
TJL01/867	**2**	11	164	175	6.286	12	156	168	7.143				
	3	16	157	173	9.246	17	158	175	9.714				
	4	19	128	147	12.925	15	135	150	10.000				
	5	23	87	110	20.910	24	96	120	20.833				
	6	10	148	158	6.329	11	134	145	7.586				
	7	17	152	169	10.059	16	156	172	9.302				
	8	11	127	138	7.971	11	123	134	8.209				
	9	9	126	135	6.667	8	134	142	5.634				
	10	9	162	171	5.263	8	163	171	4.678				
Total		**138**	**1370**	**1508**	**9.151**	**133**	**1369**	**1502**	**8.855**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	5	3	8	62.500	5	3	8	62.500				
TJL01/867	**2**	5	0	5	100.000	5	0	5	100.000				
	3	10	0	10	100.000	10	0	10	100.000				
	4	6	1	7	85.714	6	1	7	85.714				
	5	5	0	5	100.000	5	0	5	100.000				
	6	7	0	7	100.000	7	0	7	100.000				
	7	6	0	6	100.000	6	0	6	100.000				
	8	5	1	6	83.333	5	1	6	83.333				
	9	5	2	7	71.429	5	2	7	71.429				
	10	5	2	7	71.429	5	2	7	71.429				
Total		**59**	**9**	**68**	**86.765**	**59**	**9**	**68**	**86.765**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	11	177	188	5.851	10	177	187	5.348				
TJL02/848	**2**	9	133	142	6.338	9	131	140	6.429	10	127	137	7.299
	3	13	178	191	6.806	12	186	198	6.061				
	4	15	136	151	9.934	15	137	152	9.868	17	134	151	11.258
	5	5	185	190	2.632	6	180	186	3.226				
	6	17	174	191	8.901	17	174	191	8.901	16	169	185	8.649
	7	24	172	196	12.245	24	182	206	11.650				
	8	9	139	148	6.081	9	144	153	5.882	8	139	148	5.405
	9	12	169	181	6.630	12	170	182	6.593				
	10	7	177	184	3.804	7	178	185	3.784	8	172	190	4.211
Total		**122**	**1640**	**1762**	**6.924**	**121**	**1659**	**1780**	**6.798**	**59**	**752**	**811**	**7.275**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	8	2	10	80.000	8	2	10	80.000				
TJL02/848	**2**	8	0	8	100.000	8	0	8	100.000	9	0	9	100.000
	3	5	1	6	83.333	5	1	6	83.333				
	4	2	0	2	100.000	2	0	2	100.000	2	1	3	66.667
	5	5	1	6	83.333	5	1	6	83.333				
	6	4	0	4	100.000	4	0	4	100.000	4	0	4	100.000
	7	3	0	3	100.000	3	0	3	100.000				
	8	4	0	4	100.000	4	0	4	100.000	4	1	5	80.000
	9	5	1	6	83.333	5	1	6	83.333				
	10	2	0	2	100.000	2	0	2	100.000	2	0	2	100.000
Total		**46**	**5**	**51**	**90.196**	**46**	**5**	**51**	**90.196**	**21**	**2**	**23**	**91.304**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	15	251	266	5.640	15	257	272	5.515				
TJL09/962	**2**	11	165	176	6.250	10	170	180	5.556				
	3	13	137	150	8.667	13	134	147	8.844				
	4	15	161	176	8.523	13	154	167	7.784				
	5	13	170	183	7.104	12	164	176	6.818				
	6	10	243	253	3.953	8	244	252	3.175				
	7	15	200	215	7.500	14	194	208	6.731				
	8	20	222	242	8.264	19	228	247	7.692				
	9	29	246	275	10.545	30	251	281	10.676				
	10	10	166	176	5.682	10	166	276	5.682				
Total		**151**	**1961**	**2112**	**7.150**	**144**	**1962**	**2106**	**6.838**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	5	0	5	100.000	5	0	5	100.000				
TJL09/962	**2**	7	0	7	100.000	7	0	7	100.000				
	3	5	1	6	83.333	5	1	6	83.333				
	4	6	1	7	85.714	6	1	7	85.714				
	5	6	1	7	85.714	6	1	7	85.714				
	6	7	1	8	87.500	7	1	8	87.500				
	7	8	0	8	100.000	8	0	8	100.000				
	8	4	0	4	100.000	4	0	4	100.000				
	9	4	1	5	80.000	4	1	5	80.000				
	10	5	1	6	83.333	5	1	6	83.333				
Total		**57**	**6**	**63**	**90.476**	**57**	**6**	**63**	**90.476**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	12	184	196	6.122	12	183	125	6.154				
TJA09/19469	**2**	16	181	197	8.122	16	181	197	8.122	18	157	175	10.286
	3	28	189	217	12.903	28	187	215	13.023				
	4	13	141	154	8.442	13	137	150	8.667	14	146	160	8.750
	5	17	172	189	8.995	16	176	192	8.333				
	6	10	147	157	6.369	10	147	157	6.369	11	147	158	6.962
	7	15	194	209	7.177	15	196	211	7.109				
	8	12	155	167	7.186	12	156	168	7.143	12	162	174	6.897
	9	10	169	179	5.587	10	168	178	5.618				
	10	16	162	178	8.989	16	162	178	8.989	15	156	171	8.772
Total		**149**	**1694**	**1843**	**8.085**	**148**	**1693**	**1841**	**8.039**	**70**	**752**	**838**	**8.353**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	12	0	12	100.000	12	0	12	100.000				
TJA09/19469	**2**	8	0	8	100.000	8	0	8	100.000	7	1	8	87.500
	3	11	0	11	100.000	11	0	11	100.000				
	4	9	0	9	100.000	9	0	9	100.000	8	0	8	100.000
	5	8	0	8	100.000	8	0	8	100.000				
	6	8	0	8	100.000	8	0	8	100.000	10	0	10	100.000
	7	10	0	10	100.000	10	0	10	100.000				
	8	8	0	8	100.000	8	0	8	100.000	8	0	8	100.000
	9	15	0	15	100.000	15	0	15	100.000				
	10	8	0	8	100.000	8	0	8	100.000	8	0	8	100.000
Total		**97**	**0**	**97**	**100.000**	**97**	**0**	**97**	**100.000**	**41**	**1**	**42**	**97.619**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	21	90	111	18.919	22	90	112	19.643				
TJL92/093	**2**	16	111	127	12.598	17	112	129	13.178				
	3	13	96	109	11.927	13	98	111	11.712				
	4	14	100	114	12.281	14	100	114	12.281				
	5	15	110	125	12.000	15	107	122	12.295				
	6	14	131	145	9.655	14	130	144	9.722				
	7	15	116	131	11.450	14	112	126	11.111				
	8	8	120	128	6.250	8	117	125	6.400				
	9	18	115	133	13.534	18	115	133	13.534				
	10	10	106	116	8.621	10	106	116	8.621				
Total		**144**	**1095**	**1239**	**11.622**	**145**	**1087**	**1232**	**11.769**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	12	0	12	100.000	12	0	12	100.000				
TJL92/093	**2**	14	0	14	100.000	14	0	14	100.000				
	3	11	0	11	100.000	11	0	11	100.000				
	4	7	3	10	70.000	7	3	10	70.000				
	5	12	0	12	100.000	12	0	12	100.000				
	6	9	1	10	90.000	9	1	10	90.000				
	7	7	3	10	70.000	7	3	10	70.000				
	8	9	1	10	90.000	9	1	10	90.000				
	9	8	5	13	61.538	8	5	13	61.538				
	10	7	3	10	70.000	7	3	10	70.000				
Total		**96**	**16**	**112**	**85.714**	**96**	**16**	**112**	**85.714**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	13	142	155	8.387	13	149	162	8.025				
TJL88/673	**2**	6	131	137	4.380	6	131	137	4.380	8	120	128	6.250
	3	9	169	178	5.056	9	164	173	5.202				
	4	7	175	182	3.846	7	177	184	3.804	8	168	176	4.545
	5	17	174	191	8.900	17	178	195	8.718				
	6	17	177	194	8.763	17	176	193	8.808	13	172	185	7.027
	7	12	175	187	6.417	12	170	182	6.593				
	8	13	150	163	7.975	13	150	163	7.975	14	139	153	9.150
	9	7	171	178	3.933	7	169	176	3.977				
	10	8	144	152	5.263	8	144	152	5.263	8	137	145	5.517
Total		**1717**	**109**	**1608**	**6.348**	**1717**	**109**	**1608**	**6.348**	**51**	**736**	**787**	**6.480**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	1	4	5	20.000	1	4	5	20.000				
TJL88/673	**2**	1	3	4	25.000	1	3	4	25.000	1	4	5	20
	3	3	5	8	37.500	3	5	8	37.500				
	4	2	5	7	28.571	2	5	7	28.571	2	5	7	28.571
	5	2	1	3	66.667	2	1	3	66.667				
	6	4	5	9	44.444	4	5	9	44.444	4	4	8	50
	7	1	6	7	14.286	1	6	7	14.286				
	8	2	3	5	40.000	2	3	5	40.000	2	4	6	33.333
	9	2	4	6	33.333	2	4	6	33.333				
	10	1	4	5	20.000	1	4	5	20.000	1	4	5	20
Total		**19**	**40**	**59**	**32.203**	**19**	**40**	**59**	**32.203**	**10**	**21**	**31**	**32.258**

8.1.1.2 Mitf Immunoreactivity in CGCG

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	4	142	146	2.740	4	142	146	2.740				
TJL91/651	**2**	2	137	139	1.439	3	140	143	2.100				
	3	3	209	212	1.415	3	209	212	1.415				
	4	6	174	180	3.333	6	179	185	3.243				
	5	4	143	147	2.721	4	148	152	2.632				
	6	9	150	159	5.660	8	157	165	4.849				
	7	11	180	191	5.760	9	175	184	4.891				
	8	5	158	163	3.067	7	155	162	4.321				
	9	5	167	172	2.907	4	169	173	4.046				
	10	1	217	218	0.459	1	214	215	0.465				
Total		**50**	**1677**	**1727**	**2.895**	**49**	**1688**	**1737**	**2.830**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	0	0	0	0.000	0	0	0	0.000				
TJL91/651	**2**	2	0	2	100.000	2	0	2	100.000				
	3	2	0	2	100.000	2	0	2	100.000				
	4	5	0	5	100.000	5	0	5	100.000				
	5	3	0	3	100.000	3	0	3	100.000				
	6	2	0	2	100.000	2	0	2	100.000				
	7	1	0	1	100.000	1	0	1	100.000				
	8	0	2	2	0.000	0	2	2	0.000				
	9	2	0	2	100.000	2	0	2	100.000				
	10	4	0	4	100.000	4	0	4	100.000				
Total		**21**	**2**	**23**	**91.304**	**21**	**2**	**23**	**91.304**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	5	151	156	3.205	6	160	166	3.614				
TJL94/271	**2**	7	137	144	4.861	7	137	144	4.861	8	151	159	1.887
	3	11	166	177	6.215	9	163	172	5.234				
	4	9	153	162	5.556	9	153	162	5.556	7	155	162	4.348
	5	6	181	187	3.209	6	179	185	3.243				
	6	8	112	120	6.667	9	112	121	7.438	10	102	112	8.929
	7	10	185	195	5.128	8	179	187	4.470				
	8	12	123	135	8.889	12	123	135	8.889	14	124	138	10.145
	9	9	181	190	4.737	9	178	187	4.813				
	10	8	121	129	6.202	8	121	129	6.202	8	108	116	6.897
Total		**85**	**1510**	**1595**	**5.329**	**83**	**1505**	**1588**	**5.227**	**47**	**640**	**687**	**6.841**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	10	1	11	90.910	10	1	11	90.910				
TJL94/271	**2**	9	1	10	90.000	9	1	10	90.000	12	1	13	92.310
	3	6	2	8	75.000	6	2	8	75.000				
	4	7	0	7	100.000	7	0	7	100.000	7	0	7	100.000
	5	9	1	10	90.000	9	1	10	90.000				
	6	8	0	8	100.000	8	0	8	100.000	7	0	7	100.000
	7	8	0	8	100.000	8	0	8	100.000				
	8	5	0	5	100.000	5	0	5	100.000	4	0	4	100.000
	9	3	2	5	60.000	3	2	5	60.000				
	10	5	1	6	83.333	5	1	6	83.333	5	1	6	83.333
Total		**70**	**8**	**78**	**89.744**	**70**	**8**	**78**	**89.744**	**35**	**2**	**37**	**94.595**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	19	128	147	12.925	20	127	147	13.605				
TJA1018195	**2**	15	114	129	11.628	15	114	129	11.628	12	119	131	9.160
	3	18	105	123	14.634	15	108	123	12.195				
	4	14	156	170	8.235	13	157	170	7.647	17	141	158	10.759
	5	15	170	185	8.108	18	167	185	9.730				
	6	16	103	119	13.445	16	103	119	13.445	17	92	109	15.596
	7	23	171	194	11.856	22	172	194	11.340				
	8	14	98	112	12.500	14	98	112	12.500	14	86	100	14.000
	9	19	176	195	9.744	19	177	196	9.694				
	10	27	121	148	18.243	29	118	147	19.728	30	122	152	19.737
Total		**180**	**1342**	**1522**	**11.827**	**181**	**1341**	**1522**	**11.892**	**90**	**560**	**650**	**13.846**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	7	0	7	100.000	7	0	7	100.000				
TJA1018195	**2**	11	1	12	91.667	11	1	12	91.667	11	1	12	91.667
	3	6	0	6	100.000	6	0	6	100.000				
	4	7	1	8	87.500	7	1	8	87.500	6	0	6	100.000
	5	4	0	4	100.000	4	0	4	100.000				
	6	6	1	7	85.714	6	1	7	85.714	6	1	7	85.714
	7	8	0	8	100.000	8	0	8	100.000				
	8	7	0	7	100.000	7	0	7	100.000	8	0	8	100.000
	9	6	2	8	75.000	6	2	8	75.000				
	10	9	0	9	100.000	9	0	9	100.000	8	0	8	100.000
Total		**71**	**5**	**76**	**93.421**	**71**	**5**	**76**	**93.421**	**39**	**2**	**41**	**95.122**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	11	92	103	10.680	10	97	107	9.346				
TJL97/491	**2**	7	135	142	4.930	8	129	137	5.840				
	3	10	135	145	6.900	9	134	143	6.294				
	4	7	118	125	5.600	7	125	132	5.303				
	5	7	163	170	4.118	7	156	163	4.294				
	6	10	94	104	9.615	9	98	107	8.411				
	7	13	111	124	10.484	12	110	122	9.836				
	8	5	107	112	4.464	6	105	111	5.405				
	9	18	116	134	13.433	17	113	130	13.077				
	10	9	128	137	6.570	9	130	139	6.475				
Total		**97**	**1199**	**1296**	**7.485**	**94**	**1197**	**1291**	**7.281**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	9	0	9	100.000	9	0	9	100.000				
TJL97/491	**2**	7	0	7	100.000	7	0	7	100.000				
	3	3	1	4	75.000	3	1	4	75.000				
	4	8	2	10	80.000	8	2	10	80.000				
	5	7	1	8	87.500	7	1	8	87.500				
	6	10	0	10	100.000	10	0	10	100.000				
	7	4	0	4	100.000	4	0	4	100.000				
	8	3	1	4	75.000	3	1	4	75.000				
	9	6	1	7	85.710	6	1	7	85.710				
	10	9	1	10	90.000	9	1	10	90.000				
Total		**66**	**7**	**73**	**90.411**	**66**	**7**	**73**	**90.411**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	12	174	186	6.452	12	174	186	6.452				
TJL98/370	**2**	18	124	142	12.676	18	126	144	12.500	22	105	127	18.110
	3	9	156	165	5.455	9	157	166	5.423				
	4	10	123	133	7.519	10	123	133	7.519	12	123	139	11.511
	5	11	133	144	7.639	11	132	143	7.692				
	6	12	120	132	9.091	12	120	132	9.091	11	118	133	11.278
	7	12	206	218	5.769	12	206	218	5.769				
	8	14	151	165	8.485	14	151	165	8.485	15	137	152	9.868
	9	10	207	217	4.608	11	207	218	5.046				
	10	23	127	150	15.333	23	127	150	15.333	17	117	138	15.217
Total		**131**	**1521**	**1652**	**7.930**	**132**	**1523**	**1655**	**7.976**	**77**	**612**	**689**	**11.176**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	9	2	11	81.818	9	2	11	81.818				
TJL98/370	**2**	9	0	9	100.000	9	0	9	100.000	8	0	8	100.000
	3	16	0	16	100.000	16	0	16	100.000				
	4	12	0	12	100.000	12	0	12	100.000	11	0	11	100.000
	5	13	0	13	100.000	13	0	13	100.000				
	6	7	4	11	63.636	7	4	11	63.636	8	3	11	72.728
	7	9	2	11	81.818	9	2	11	81.818				
	8	6	6	12	50.000	6	6	12	50.000	10	0	10	100.000
	9	7	3	10	70.000	7	3	10	70.000				
	10	6	0	6	100.000	6	0	6	100.000	6	0	6	100.000
Total		**94**	**17**	**111**	**84.685**	**94**	**17**	**111**	**84.685**	**43**	**3**	**46**	**93.478**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	26	114	140	18.571	26	113	139	18.705				
TJL02/068	**2**	22	175	197	11.168	22	173	195	11.282				
	3	21	200	221	9.502	23	201	224	10.268				
	4	19	207	226	8.407	19	209	228	8.333				
	5	22	217	239	9.205	22	216	238	9.244				
	6	19	210	229	8.297	19	210	229	8.297				
	7	18	200	218	8.257	18	200	218	8.257				
	8	24	177	201	11.940	23	179	202	11.386				
	9	16	206	222	7.207	17	203	220	7.727				
	10	18	260	278	6.475	18	261	279	6.452				
Total		**205**	**1966**	**2171**	**9.443**	**207**	**1965**	**2172**	**9.530**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	4	0	4	100.000	4	0	4	100.000				
TJL02/06	**2**	9	0	9	100.000	9	0	9	100.000				
	3	9	0	9	100.000	9	0	9	100.000				
	4	11	0	11	100.000	11	0	11	100.000				
	5	6	0	6	100.000	6	0	6	100.000				
	6	10	0	10	100.000	10	0	10	100.000				
	7	6	0	6	100.000	6	0	6	100.000				
	8	4	0	4	100.000	4	0	4	100.000				
	9	7	0	7	100.000	7	0	7	100.000				
	10	8	0	8	100.000	8	0	8	100.000				
Total		**74**	**0**	**74**	**100.000**	**74**	**0**	**74**	**100.000**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	17	99	116	14.655	17	98	115	14.783				
TJL91/581	**2**	3	117	120	2.500	3	118	121	2.479	4	113	117	3.419
	3	5	134	139	3.597	6	136	142	4.225				
	4	6	94	100	6.000	6	91	97	6.186	8	86	94	8.511
	5	5	139	144	3.472	5	138	143	3.497				
	6	8	85	93	8.602	8	85	93	8.602	8	84	92	8.696
	7	11	95	106	10.377	11	95	106	10.378				
	8	6	48	54	11.111	6	47	53	11.321	5	40	45	11.111
	9	1	105	106	0.943	2	102	104	1.923				
	10	6	52	58	10.345	6	51	57	10.526	7	47	54	12.963
Total		**68**	**968**	**1036**	**6.564**	**70**	**961**	**1031**	**6.790**	**32**	**370**	**402**	**8.940**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	5	0	5	100.000	5	0	5	100.000				
TJL91/581	**2**	1	3	4	25.000	1	3	4	25.000	1	2	3	33.333
	3	2	5	7	28.571	2	5	7	28.571				
	4	1	2	3	33.333	1	2	3	33.333	1	1	2	50.000
	5	1	6	7	14.286	1	6	7	14.286				
	6	4	3	7	57.143	4	3	7	57.143	7	1	8	87.500
	7	3	2	5	60.000	3	2	5	60.000				
	8	6	4	10	60.000	6	4	10	60.000	5	6	11	45.455
	9	0	7	7	0.000	0	7	7	0.000				
	10	4	0	4	100.000	4	0	4	100.000	4	1	5	80.000
Total		**27**	**32**	**59**	**45.763**	**27**	**32**	**59**	**45.763**	**18**	**11**	**29**	**62.069**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	7	170	177	3.955	7	170	177	3.955				
TJL90/409	**2**	6	131	137	4.380	6	132	138	4.348				
	3	7	241	248	2.823	7	240	247	2.834				
	4	6	169	175	3.429	6	169	175	3.429				
	5	5	147	152	3.289	5	148	153	3.268				
	6	1	162	163	0.613	1	162	163	0.613				
	7	4	153	157	2.548	4	151	155	2.581				
	8	3	160	163	1.840	3	160	163	1.840				
	9	5	200	205	2.439	5	198	203	2.463				
	10	7	250	257	2.724	7	246	253	2.767				
Total		**51**	**1783**	**1834**	**2.781**	**51**	**1776**	**1827**	**2.791**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	5	1	6	83.333	5	1	6	83.333				
TJL90/409	**2**	6	2	8	75.000	6	2	8	75.000				
	3	2	2	4	50.000	2	2	4	50.000				
	4	3	5	8	37.500	3	5	8	37.500				
	5	4	3	7	57.143	4	3	7	57.143				
	6	3	3	6	50.000	3	3	6	50.000				
	7	10	0	10	100.000	10	0	10	100.000				
	8	6	3	9	66.667	6	3	9	66.667				
	9	6	4	10	60.000	6	4	10	60.000				
	10	4	4	8	50.000	4	4	8	50.000				
Total		**49**	**27**	**76**	**64.474**	**49**	**27**	**76**	**64.474**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	7	63	70	10.000	7	64	71	9.859				
TJL09/075	**2**	6	32	38	18.750	6	31	37	16.216	5	33	38	13.158
	3	3	94	97	3.093	3	96	99	3.030				
	4	5	67	72	6.944	5	67	72	6.944	7	60	67	10.448
	5	6	29	35	17.143	6	27	33	18.182				
	6	11	68	79	13.924	11	68	79	13.924	12	61	73	16.438
	7	9	112	121	7.438	9	107	116	7.759				
	8	7	100	107	6.542	7	98	105	6.667	11	96	107	10.280
	9	8	90	98	8.163	8	90	98	8.163				
	10	6	31	37	16.216	6	31	37	16.216	5	28	33	15.152
Total		**68**	**686**	**754**	**9.019**	**68**	**679**	**747**	**9.103**	**40**	**278**	**318**	**12.579**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	1	0	1	100.000	1	0	1	100.000				
TJL90/075	**2**	1	0	1	100.000	1	0	1	100.000	1	0	1	100.000
	3	1	1	2	50.000	1	1	2	50.000				
	4	1	0	1	100.000	1	0	1	100.000	1	0	1	100.000
	5	0	2	2	0.000	0	2	2	0.000				
	6	2	1	3	66.667	2	1	3	66.667	2	1	3	66.667
	7	4	2	6	66.667	4	2	6	66.667				
	8	2	0	2	100.000	2	0	2	100.000	2	1	3	66.667
	9	1	2	3	33.333	1	2	3	33.333				
	10	2	0	2	100.000	2	0	2	100.000	2	1	3	66.667
Total		**15**	**8**	**23**	**65.217**	**15**	**8**	**23**	**65.217**	**8**	**3**	**11**	**72.727**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	16	208	224	7.143	16	207	223	7.175				
TJL89/488	**2**	12	198	210	5.714	12	196	208	5.769				
	3	10	86	96	10.417	10	89	99	10.101				
	4	5	78	83	6.024	5	80	85	5.882				
	5	4	117	121	3.306	4	116	120	3.333				
	6	7	107	114	6.140	7	107	114	6.140				
	7	11	168	179	6.145	11	168	179	6.145				
	8	9	148	157	5.732	9	149	158	5.696				
	9	9	122	131	6.870	9	120	129	6.977				
	10	6	138	144	4.167	6	139	145	4.138				
Total		**89**	**1370**	**1459**	**6.100**	**89**	**1371**	**1460**	**6.096**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	6	5	11	54.545	6	5	11	54.545				
TJL89/488	**2**	2	5	7	28.571	2	5	7	28.571				
	3	1	1	2	50.000	1	1	2	50.000				
	4	5	1	6	83.333	5	1	6	83.333				
	5	1	2	3	33.333	1	2	3	33.333				
	6	3	1	4	75.000	3	1	4	75.000				
	7	3	2	5	60.000	3	2	5	60.000				
	8	5	0	5	100.000	5	0	5	100.000				
	9	3	5	8	37.500	3	5	8	37.500				
	10	0	6	6	0.000	0	6	6	0.000				
Total		**29**	**28**	**57**	**50.877**	**29**	**28**	**57**	**50.877**				

8.1.1.3 Mitf Immunoreactivity in cherubism

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	11	110	121	9.091	11	111	122	9.016				
TJL03/887	**2**	17	101	118	14.407	16	102	118	13.559	18	109	127	14.173
	3	16	128	144	11.111	16	128	144	11.111				
	4	8	108	116	6.897	8	108	116	6.897	7	102	109	6.422
	5	7	120	127	5.512	7	120	127	5.833				
	6	18	137	155	11.613	18	137	155	11.613	20	137	157	12.739
	7	6	112	118	5.085	6	113	119	5.042				
	8	10	134	144	6.944	9	131	140	6.429	10	132	142	7.042
	9	16	118	134	11.940	16	121	137	11.679				
	10	13	98	111	11.712	13	99	112	11.607	12	97	109	11.009
Total		**122**	**1166**	**1288**	**9.472**	**120**	**1170**	**1290**	**9.302**	**67**	**577**	**644**	**10.404**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	1	6	0	6	100.000	6	0	6	100.000				
TJL03/887	2	8	0	8	100.000	8	0	8	100.000	8	1	9	88.889
	3	9	2	11	81.818	9	2	11	81.818				
	4	11	1	12	91.667	11	1	12	91.667	11	1	12	91.667
	5	10	1	11	90.909	10	1	11	90.909				
	6	8	0	8	100.000	8	0	8	100.000	8	0	8	100
	7	9	0	9	100.000	9	0	9	100.000				
	8	5	0	5	100.000	5	0	5	100.000	6	0	6	100
	9	14	2	16	87.500	14	2	16	87.500				
	10	14	1	15	93.333	14	1	15	93.333	12	1	13	92.310
Total		94	7	101	93.069	94	7	101	93.069	45	3	48	93.750

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	1	7	96	103	6.796	6	96	102	5.882				
TJL95/763	2	7	181	188	3.723	8	170	178	4.706				
	3	3	102	105	2.857	4	107	111	3.604				
	4	0	74	74	0.000	0	76	76	0.000				
	5	2	136	138	1.450	2	133	135	1.481				
	6	2	130	132	1.515	2	123	125	1.600				
	7	5	154	159	3.145	5	143	148	3.378				
	8	5	114	119	4.202	4	111	115	3.478				
	9	6	132	138	4.348	6	127	133	4.511				
	10	2	112	114	1.754	2	115	117	1.709				
Total		39	1231	1270	3.071	39	1201	1240	3.145				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	1	4	0	4	100.000	4	0	4	100.000				
TJL95/763	2	6	1	7	85.714	6	1	7	85.714				
	3	2	1	3	66.667	2	1	3	66.667				
	4	2	0	2	100.000	2	0	2	100.000				
	5	5	0	5	100.000	5	0	5	100.000				
	6	5	0	5	100.000	5	0	5	100.000				
	7	5	3	8	62.500	5	3	8	62.500				
	8	3	0	3	100.000	3	0	3	100.000				
	9	4	0	4	100.000	4	0	4	100.000				
	10	1	3	4	25.000	1	3	4	25.000				
Total		37	8	45	82.222	37	8	45	82.222				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	7	136	143	4.895	7	137	144	4.861				
TJL96/188	**2**	6	147	153	3.922	6	148	154	3.896				
	3	7	134	141	4.965	8	135	143	5.594				
	4	4	153	157	2.548	5	153	158	3.165				
	5	5	164	169	2.959	5	167	172	2.907				
	6	4	170	174	2.299	4	170	174	2.299				
	7	3	118	121	2.479	3	118	121	2.479				
	8	10	138	148	6.757	10	140	150	6.667				
	9	4	127	131	3.053	5	128	133	3.759				
	10	7	137	144	4.861	7	133	140	5.000				
Total		**57**	**1424**	**1481**	**3.849**	**60**	**1429**	**1489**	**4.030**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	3	0	3	100.000	3	0	3	100.000				
TJL96/188	**2**	3	0	3	100.000	3	0	3	100.000				
	3	3	0	3	100.000	3	0	3	100.000				
	4	6	0	6	100.000	6	0	6	100.000				
	5	3	1	4	75.000	3	1	4	75.000				
	6	1	4	5	20.000	1	4	5	20.000				
	7	2	0	2	100.000	2	0	2	100.000				
	8	4	1	5	80.000	4	1	5	80.000				
	9	3	1	4	75.000	3	1	4	75.000				
	10	3	1	4	75.000	3	1	4	75.000				
Total		**31**	**8**	**39**	**79.487**	**31**	**8**	**39**	**79.487**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	3	113	116	2.586	3	113	116	2.586				
TJL99/665	**2**	2	47	49	4.082	2	48	50	4.000	1	56	57	1.754
	3	2	105	107	1.869	2	107	109	1.835				
	4	1	46	47	2.128	1	46	47	2.128	0	48	48	0.000
	5	2	125	127	1.575	2	122	124	1.613				
	6	4	63	67	5.970	4	63	67	6.250	2	73	75	2.667
	7	5	114	119	4.202	5	114	119	4.202				
	8	4	75	79	5.063	4	74	78	5.128	4	80	84	4.762
	9	2	138	140	1.429	2	136	138	1.449				
	10	6	89	95	6.316	6	91	97	6.186	6	76	82	7.317
Total		**31**	**915**	**946**	**3.277**	**31**	**914**	**945**	**3.280**	**13**	**333**	**346**	**3.757**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	7	1	8	87.500	7	1	8	87.500				
TJL99/665	**2**	4	1	5	80.000	4	1	5	80.000	4	1	5	80.000
	3	4	1	5	80.000	4	1	5	80.000				
	4	1	1	2	50.000	1	1	2	50.000	1	1	2	50.000
	5	2	1	3	66.667	2	1	3	66.667				
	6	3	0	3	100.000	3	0	3	100.000	3	0	3	100.000
	7	4	3	7	57.143	4	3	7	57.143				
	8	4	0	4	100.000	4	0	4	100.000	4	0	4	100.000
	9	3	0	3	100.000	3	0	3	100.000				
	10	6	1	7	85.714	6	1	7	85.714	7	1	8	87.500
Total		**38**	**9**	**47**	**80.851**	**38**	**9**	**47**	**80.851**	**19**	**3**	**22**	**86.364**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	4	142	146	2.740	4	141	145	2.759				
TJL99/223	**2**	6	91	95	6.316	6	88	94	6.383	3	101	104	2.885
	3	3	120	123	2.439	3	121	124	2.419				
	4	5	92	97	5.155	5	92	97	5.155	4	79	83	4.819
	5	5	148	153	3.268	5	149	154	3.247				
	6	5	83	88	5.682	5	82	87	5.747	3	82	85	3.529
	7	2	134	136	1.471	2	134	136	1.471				
	8	3	136	139	2.158	3	134	137	2.190	2	139	141	1.418
	9	4	128	132	3.030	4	128	132	3.030				
	10	6	95	101	5.941	6	95	101	5.941	4	94	98	4.082
Total		**43**	**1167**	**1210**	**3.554**	**43**	**1164**	**1207**	**3.563**	**16**	**495**	**511**	**3.131**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	5	7	12	41.667	5	7	12	41.667				
TJL99/223	**2**	10	4	14	71.429	10	4	14	71.429	10	6	16	62.500
	3	3	4	7	42.857	3	4	7	42.857				
	4	6	3	9	66.667	6	3	9	66.667	7	4	11	63.636
	5	1	3	4	25.000	1	3	4	25.000				
	6	7	2	9	77.778	7	2	9	77.778	6	4	10	60.000
	7	0	7	7	0.000	0	7	7	0.000				
	8	2	12	14	14.286	2	12	14	14.286	4	8	12	33.333
	9	2	4	6	33.333	2	4	6	33.333				
	10	8	0	8	100.000	8	0	8	100.000	7	1	8	87.500
Total		**44**	**46**	**90**	**48.889**	**44**	**46**	**90**	**48.889**	**34**	**23**	**57**	**59.649**

8.1.1.4 Mitf Immunoreactivity in ABC

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	4	121	125	3.200	4	124	128	3.125				
TJL88/279	**2**	3	137	140	2.143	3	137	140	2.143	2	153	156	1.282
	3	3	193	196	1.531	3	193	196	1.531				
	4	4	111	115	3.478	5	112	117	4.274	3	112	115	2.609
	5	1	257	258	0.388	1	248	249	0.402				
	6	3	105	108	2.778	3	107	110	2.727	2	102	104	1.923
	7	3	150	153	1.961	3	151	154	1.948				
	8	7	134	141	4.965	7	134	141	4.965	6	130	136	4.412
	9	4	194	198	2.020	4	197	201	1.990				
	10	7	144	151	4.636	7	144	151	4.636	8	147	155	5.161
Total		**39**	**1546**	**1585**	**2.461**	**40**	**1547**	**1587**	**2.520**	**21**	**645**	**666**	**3.153**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	2	3	5	40.000	2	3	5	40.000				
TJL88/279	**2**	1	2	3	33.333	1	2	3	33.333	1	2	3	33.333
	3	1	4	5	20.000	1	4	5	20.000				
	4	1	0	1	100.000	1	0	1	100.000	1	0	1	100.000
	5	0	3	3	0.000	0	3	3	0.000				
	6	1	3	4	25.000	1	3	4	25.000	3	1	4	75.000
	7	1	4	5	20.000	1	4	5	20.000				
	8	2	1	3	66.667	2	1	3	66.667	2	1	3	66.667
	9	2	3	5	40.000	2	3	5	40.000				
	10	3	0	3	100.000	3	0	3	100.000	2	0	2	100.000
Total		**14**	**23**	**37**	**37.838**	**14**	**23**	**37**	**37.838**	**9**	**4**	**13**	**69.231**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	2	136	138	1.449	2	134	136	1.471				
TJL88/593	**2**	1	119	120	0.833	1	113	114	0.877	1	111	112	0.893
	3	2	115	117	1.709	2	117	119	1.681				
	4	0	106	106	0.000	0	106	106	0.000	0	101	101	0.000
	5	2	121	123	1.626	2	121	123	1.626				
	6	2	107	109	1.835	3	108	111	2.703	1	104	105	0.952
	7	3	151	154	1.948	3	154	157	1.911				
	8	2	130	132	1.515	2	128	130	1.538	2	136	138	1.449
	9	2	126	128	1.563	2	126	128	1.563				
	10	4	106	110	3.636	3	99	102	2.941	6	126	132	4.545
Total		**20**	**1217**	**1237**	**1.617**	**20**	**1206**	**1226**	**1.631**	**10**	**578**	**588**	**1.701**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	1	2	3	33.333	1	2	3	33.333				
TJL88/593	**2**	0	4	4	0.000	0	4	4	0.000	0	4	4	0.000
	3	0	2	2	0.000	0	2	2	0.000				
	4	0	4	4	0.000	0	4	4	0.000	0	4	4	0.000
	5	1	2	3	33.333	1	2	3	33.333				
	6	0	5	5	0.000	0	5	5	0.000	0	5	5	0.000
	7	0	4	4	0.000	0	4	4	0.000				
	8	1	1	2	50.000	1	1	2	50.000	1	1	2	50.000
	9	1	1	2	50.000	1	1	2	50.000				
	10	0	1	1	0.000	0	1	1	0.000	0	1	1	0.000
Total		**4**	**26**	**30**	**13.333**	**4**	**26**	**30**	**13.333**	**1**	**15**	**16**	**6.250**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	3	155	158	1.899	3	149	152	1.974				
TJL99/489	**2**	4	134	138	2.896	4	139	143	2.798				
	3	3	141	144	2.083	3	138	141	2.128				
	4	3	173	176	1.705	3	170	173	1.734				
	5	11	183	194	5.670	10	187	197	5.076				
	6	2	137	139	1.439	2	141	143	1.399				
	7	3	128	131	2.290	3	135	138	2.174				
	8	5	128	133	3.760	5	126	131	3.817				
	9	6	192	198	3.030	6	184	190	3.158				
	10	4	150	154	2.597	4	157	161	2.484				
Total		**44**	**1521**	**1565**	**2.812**	**43**	**1526**	**1569**	**2.741**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	5	0	5	100.000	5	0	5	100.000				
TJL99/489	**2**	8	0	8	100.000	8	0	8	100.000				
	3	5	0	5	100.000	5	0	5	100.000				
	4	6	0	6	100.000	6	0	6	100.000				
	5	5	1	6	83.333	5	1	6	83.333				
	6	5	1	6	83.333	5	1	6	83.333				
	7	5	0	5	100.000	5	0	5	100.000				
	8	7	0	7	100.000	7	0	7	100.000				
	9	4	0	4	100.000	4	0	4	100.000				
	10	3	1	4	75.000	3	1	4	75.000				
Total		**53**	**3**	**56**	**94.643**	**53**	**3**	**56**	**94.643**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	7	84	91	7.692	7	86	93	7.527				
TJA09/37588	**2**	4	89	93	4.301	4	89	93	4.301				
	3	5	104	109	4.587	5	102	107	4.673				
	4	4	93	97	4.124	4	93	97	4.124				
	5	7	114	121	5.785	7	112	119	5.882				
	6	6	127	133	4.511	6	126	132	4.545				
	7	7	115	122	5.738	7	115	122	5.738				
	8	7	126	133	5.263	7	124	131	5.344				
	9	5	112	117	4.274	5	112	117	4.274				
	10	6	118	124	4.839	6	120	126	4.762				
Total		**58**	**1082**	**1140**	**5.088**	**58**	**1079**	**1137**	**5.101**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	1	8	9	11.111	1	8	9	11.111				
TJA09/37588	**2**	1	6	7	14.286	1	6	7	14.286				
	3	0	6	6	0.000	0	6	6	0.000				
	4	1	5	6	16.667	1	5	6	16.667				
	5	1	4	5	20.000	1	4	5	20.000				
	6	1	6	7	14.286	1	6	7	14.286				
	7	0	6	6	0.000	0	6	6	0.000				
	8	1	4	5	20.000	1	4	5	20.000				
	9	0	8	8	0.000	0	8	8	0.000				
	10	1	6	7	14.286	1	6	7	14.286				
Total		**7**	**59**	**66**	**10.606**	**7**	**59**	**66**	**10.606**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	2	145	147	1.361	2	141	143	1.400				
TJL87/725	**2**	4	139	143	2.800	4	141	145	2.759				
	3	5	105	110	4.545	5	105	110	4.545				
	4	3	152	155	1.935	4	154	158	2.532				
	5	0	114	114	0.000	0	114	114	0.000				
	6	2	87	89	2.247	2	87	89	2.247				
	7	4	106	110	3.636	4	104	108	3.704				
	8	1	111	112	0.893	1	112	113	0.885				
	9	5	98	103	4.854	3	94	97	3.093				
	10	4	54	58	6.897	4	54	58	6.897				
Total		**30**	**1111**	**1141**	**2.629**	**29**	**1106**	**1135**	**2.555**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	3	0	3	100.000	3	0	3	100.000				
TJL87/725	**2**	2	0	2	100.000	2	0	2	100.000				
	3	5	0	5	100.000	5	0	5	100.000				
	4	3	0	3	100.000	3	0	3	100.000				
	5	2	0	2	100.000	2	0	2	100.000				
	6	0	2	2	0.000	0	2	2	0.000				
	7	1	1	2	50.000	1	1	2	50.000				
	8	2	0	2	100.000	2	0	2	100.000				
	9	1	0	1	100.000	1	0	1	100.000				
	10	3	0	3	100.000	3	0	3	100.000				
Total		**22**	**3**	**25**	**88.000**	**22**	**3**	**25**	**88.000**				

8.1.2 Tfe3 Immunoreactivity

8.1.2.1 Tfe3 Immunoreactivity in PGCG

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	2	187	189	1.058	2	183	185	1.081				
TJL91/195	**2**	28	99	127	22.047	28	99	127	22.047	34	88	122	27.869
	3	2	171	173	1.156	2	172	174	1.149				
	4	18	92	110	16.364	17	93	110	15.455	22	103	125	17.600
	5	3	231	234	1.282	3	225	228	1.316				
	6	11	123	134	8.209	11	121	132	8.333	13	131	144	9.028
	7	1	147	148	0.676	1	148	149	0.671				
	8	26	121	147	17.687	26	121	147	17.687	26	112	138	18.841
	9	1	133	134	0.746	1	132	133	0.752				
	10	28	120	148	18.919	27	121	148	18.243	28	118	146	19.178
Total		**120**	**1424**	**1544**	**7.772**	**118**	**1415**	**1533**	**7.697**	**123**	**552**	**675**	**18.222**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	2	2	4	50.000	2	2	4	50.000				
TJL91/195	**2**	3	3	6	50.000	3	3	6	50.000	3	2	5	60.000
	3	2	5	7	28.571	2	5	7	28.571				
	4	5	3	7	71.429	5	3	7	71.429	5	3	8	62.500
	5	3	4	7	42.857	3	4	7	42.857				
	6	6	2	8	75.000	6	2	8	75.000	6	2	8	75.000
	7	0	13	13	0.000	0	13	13	0.000				
	8	4	0	4	100.000	4	0	4	100.000	4	0	4	100.000
	9	3	5	8	37.500	3	5	8	37.500				
	10	5	0	5	100.000	5	0	5	100.000	5	0	5	100.000
Total		**33**	**36**	**69**	**47.826**	**33**	**36**	**69**	**47.826**	**23**	**7**	**30**	**76.667**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	3	212	215	1.395	3	214	217	1.382				
TJL97/469	**2**	8	83	91	8.791	8	88	96	9.375				
	3	13	103	116	11.207	13	108	121	10.744				
	4	13	154	167	7.784	14	158	172	8.140				
	5	12	199	211	5.687	11	203	214	5.140				
	6	11	184	195	5.641	10	188	198	5.051				
	7	12	154	166	7.229	12	160	172	6.977				
	8	3	183	186	1.613	3	181	184	1.630				
	9	2	203	205	0.976	4	207	211	1.896				
	10	5	143	148	3.378	5	148	153	3.268				
Total		**82**	**1618**	**1700**	**4.824**	**83**	**1655**	**1738**	**4.776**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	0	6	6	0.000	0	6	6	0.000				
TJL97/469	**2**	3	1	4	75.000	3	1	4	75.000				
	3	6	1	7	85.714	6	1	7	85.714				
	4	5	0	5	100.000	5	0	5	100.000				
	5	6	1	7	85.714	6	1	7	85.714				
	6	3	3	6	50.000	3	3	6	50.000				
	7	4	0	4	100.000	4	0	4	100.000				
	8	3	1	4	75.000	3	1	4	75.000				
	9	1	5	6	16.667	1	5	6	16.667				
	10	4	3	7	57.143	4	3	7	57.143				
Total		**35**	**21**	**56**	**62.500**	**35**	**21**	**56**	**62.500**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	3	245	248	1.210	4	248	252	1.587				
TJL99/470	**2**	3	196	199	1.508	3	209	212	1.415				
	3	6	140	146	4.110	6	144	150	4.000				
	4	7	178	185	3.784	7	178	185	3.784				
	5	3	166	169	1.775	3	169	172	1.744				
	6	4	170	174	2.299	3	177	180	1.667				
	7	5	131	136	3.676	5	131	136	3.676				
	8	5	200	205	2.439	5	206	211	2.370				
	9	1	177	178	0.562	1	171	172	0.581				
	10	6	243	249	2.410	5	248	253	1.976				
Total		**43**	**1846**	**1889**	**2.276**	**42**	**1881**	**1923**	**2.184**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	0	4	4	0.000	0	4	4	0.000				
TJL99/470	**2**	0	3	3	0.000	0	3	3	0.000				
	3	0	6	6	0.000	0	6	6	0.000				
	4	0	5	5	0.000	0	5	5	0.000				
	5	0	5	5	0.000	0	5	5	0.000				
	6	0	6	6	0.000	0	6	6	0.000				
	7	0	4	4	0.000	0	4	4	0.000				
	8	0	6	6	0.000	0	6	6	0.000				
	9	0	7	7	0.000	0	7	7	0.000				
	10	0	7	7	0.000	0	7	7	0.000				
Total		**0**	**53**	**53**	**0.000**	**0**	**53**	**53**	**0.000**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	9	143	152	5.921	8	149	157	5.096				
TJL00/312	**2**	6	167	173	3.468	6	167	173	3.468	6	170	176	3.409
	3	11	120	131	8.397	10	123	133	7.519				
	4	6	93	99	6.061	6	92	98	6.122	12	116	128	9.375
	5	7	99	106	6.604	7	99	106	6.604				
	6	5	97	102	4.902	5	92	97	5.155	6	97	103	5.825
	7	8	118	126	6.349	8	118	126	6.349				
	8	13	130	143	9.091	12	131	143	8.392	9	139	148	6.081
	9	8	145	153	5.229	8	147	155	5.161				
	10	4	148	152	2.632	4	148	152	2.632	5	158	163	3.067
Total		**77**	**1260**	**1337**	**5.759**	**74**	**1266**	**1340**	**5.522**	**38**	**680**	**718**	**5.292**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	14	0	14	100.000	14	0	14	100.000				
TJL00/312	**2**	7	2	9	77.778	7	2	9	77.778	7	3	10	70.000
	3	8	0	8	100.000	8	0	8	100.000				
	4	6	3	9	66.667	6	3	9	66.667	9	1	10	90.000
	5	8	0	8	100.000	8	0	8	100.000				
	6	5	1	6	83.333	5	1	6	83.333	5	1	6	83.333
	7	8	1	9	88.889	8	1	9	88.889				
	8	6	1	7	85.714	6	1	7	85.714	6	0	6	100.000
	9	11	0	11	100.000	11	0	11	100.000				
	10	2	1	3	66.667	2	1	3	66.667	1	2	3	66.667
Total		**75**	**9**	**84**	**89.286**	**75**	**9**	**84**	**89.286**	**28**	**7**	**35**	**82.857**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	10	121	131	7.634	12	119	131	9.160				
TJL01/867	**2**	5	157	162	3.086	5	161	166	3.012				
	3	13	123	136	9.559	12	123	135	8.889				
	4	14	174	188	7.447	14	174	188	7.447				
	5	13	143	156	8.333	13	143	156	8.333				
	6	14	110	124	11.290	14	110	124	11.290				
	7	4	126	130	3.077	4	127	131	3.053				
	8	10	175	185	5.405	9	176	185	4.865				
	9	6	137	143	4.196	6	138	144	4.167				
	10	8	148	156	5.128	8	149	157	5.096				
Total		**97**	**1414**	**1511**	**6.420**	**97**	**1420**	**1517**	**6.394**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	5	5	10	50.000	5	5	10	50.000				
TJL01/867	**2**	4	5	9	44.444	4	5	9	44.444				
	3	3	4	7	42.857	3	4	7	42.857				
	4	6	0	6	100.000	6	0	6	100.000				
	5	4	3	7	57.143	4	3	7	57.143				
	6	4	7	11	36.364	4	7	11	36.364				
	7	1	5	6	16.667	1	5	6	16.667				
	8	3	5	8	37.500	3	5	8	37.500				
	9	4	4	8	50.000	4	4	8	50.000				
	10	2	3	5	40.000	2	3	5	40.000				
Total		**36**	**41**	**77**	**46.753**	**36**	**41**	**77**	**46.753**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	9	178	187	4.813	9	178	187	4.813				
TJL02/848	**2**	7	133	140	5.000	7	130	137	5.109	7	106	113	6.195
	3	9	136	145	6.207	9	137	146	6.164				
	4	13	131	144	9.028	13	131	144	9.018	11	138	149	7.383
	5	6	179	185	3.243	6	179	185	3.243				
	6	10	139	149	6.711	10	142	152	6.579	8	130	138	5.797
	7	4	237	241	1.660	4	237	241	1.660				
	8	10	140	150	6.667	10	140	150	6.667	8	154	162	4.938
	9	5	203	208	2.404	5	202	207	2.415				
	10	5	172	177	2.825	5	174	179	2.793	7	174	181	3.867
Total		**78**	**1648**	**1726**	**4.519**	**78**	**1650**	**1728**	**4.514**	**41**	**702**	**743**	**5.518**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	9	3	12	75.000	9	3	12	75.000				
TJL02/848	**2**	12	1	13	92.308	12	1	13	92.308	11	2	13	84.615
	3	9	4	13	69.231	9	4	13	69.231				
	4	9	1	10	90.000	9	1	10	90.000	10	1	11	90.910
	5	8	6	14	57.143	8	6	14	57.143				
	6	7	2	9	77.778	7	2	9	77.778	9	1	10	90.000
	7	0	11	11	0.000	0	11	11	0.000				
	8	4	0	4	100.000	4	0	4	100.000	4	0	4	100.000
	9	3	11	14	21.429	3	11	14	21.429				
	10	5	2	7	71.429	5	2	7	71.429	5	0	5	100.000
Total		**66**	**41**	**107**	**61.682**	**66**	**41**	**107**	**61.682**	**39**	**4**	**43**	**90.698**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	17	188	205	8.293	17	186	203	8.374				
TJL09/962	**2**	12	184	196	6.122	12	184	196	6.122				
	3	8	178	186	4.301	8	178	186	4.301				
	4	10	180	190	5.263	10	181	191	5.236				
	5	12	176	188	6.383	12	175	187	6.417				
	6	6	105	111	5.405	6	106	112	5.357				
	7	16	182	198	8.081	16	185	201	7.960				
	8	15	159	174	8.621	15	158	173	8.671				
	9	7	152	159	4.403	7	152	159	4.403				
	10	14	152	166	8.434	14	152	166	8.434				
Total		**117**	**1656**	**1773**	**6.599**	**117**	**1657**	**1774**	**6.595**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	2	7	9	22.222	2	7	9	22.222				
TJL09/962	**2**	5	7	12	41.667	5	7	12	41.667				
	3	2	14	16	12.500	2	14	16	12.500				
	4	2	13	15	13.333	2	13	15	13.333				
	5	4	4	8	50.000	4	4	8	50.000				
	6	3	12	15	20.000	3	12	15	20.000				
	7	2	7	9	22.222	2	7	9	22.222				
	8	3	7	10	30.000	3	7	10	30.000				
	9	3	8	11	27.273	3	8	11	27.273				
	10	2	8	10	20.000	2	8	10	20.000				
Total		**28**	**87**	**115**	**24.348**	**28**	**87**	**115**	**24.348**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	16	169	185	8.649	16	169	185	8.469				
TJA09/19469	**2**	31	348	379	8.179	30	345	375	8.000	30	338	368	8.152
	3	15	176	191	7.853	15	177	192	7.813				
	4	17	258	275	6.182	18	264	282	6.383	21	254	275	7.636
	5	19	190	209	9.091	19	189	208	9.135				
	6	33	398	431	7.657	33	392	425	7.765	30	396	426	7.042
	7	16	162	178	8.989	16	158	174	9.195				
	8	12	152	164	11.111	12	152	164	7.317	14	168	182	7.692
	9	21	168	189	11.111	21	172	193	10.881				
	10	17	166	183	9.290	17	166	183	9.290	22	168	190	11.579
Total		**197**	**2187**	**2384**	**8.263**	**197**	**2184**	**2381**	**8.274**	**117**	**1324**	**1441**	**8.119**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	7	5	12	58.333	7	5	12	58.333				
TJA09/19469	**2**	4	2	6	66.667	4	2	6	66.667	4	2	6	66.667
	3	4	4	8	50.000	4	4	8	50.000				
	4	3	4	7	42.857	3	4	7	42.857	3	4	7	42.857
	5	5	7	12	41.667	5	7	12	41.667				
	6	5	3	8	62.500	5	3	8	62.500	6	3	9	66.667
	7	6	5	11	54.545	6	5	11	54.545				
	8	6	1	7	85.714	6	1	7	85.714	4	1	5	80.000
	9	4	7	11	36.364	4	7	11	36.364				
	10	1	2	3	33.333	1	2	3	33.333	1	1	2	50.000
Total		**45**	**40**	**85**	**52.941**	**45**	**40**	**85**	**52.941**	**18**	**11**	**29**	**62.070**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	8	135	143	5.594	8	135	143	5.594				
TJL92/093	**2**	5	109	114	4.386	5	111	116	4.310				
	3	2	110	112	1.786	2	108	110	1.818				
	4	4	137	141	2.837	4	137	141	2.837				
	5	6	159	165	3.634	6	161	167	3.593				
	6	3	112	115	2.609	3	110	113	2.655				
	7	4	114	118	3.390	4	114	118	3.390				
	8	3	119	122	2.459	3	119	122	2.459				
	9	6	124	130	4.615	6	125	131	4.580				
	10	7	110	177	5.983	7	112	119	5.882				
Total		**48**	**1229**	**1277**	**3.759**	**48**	**1232**	**1280**	**3.750**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	2	9	11	18.182	2	9	11	18.182				
TJL92/093	**2**	5	6	11	45.455	5	6	11	45.455				
	3	5	8	13	38.462	5	8	13	38.462				
	4	3	6	9	33.333	3	6	9	33.333				
	5	0	10	10	0.000	0	10	10	0.000				
	6	2	9	11	18.182	2	9	11	18.182				
	7	4	8	12	33.333	4	8	12	33.333				
	8	4	8	12	33.333	4	8	12	33.333				
	9	4	8	12	33.333	3	9	12	25.000				
	10	3	6	9	33.333	3	6	9	33.333				
Total		**32**	**78**	**110**	**29.091**	**31**	**79**	**110**	**28.182**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	27	158	185	14.595	26	165	191	13.613				
TJL88/673	**2**	27	191	218	12.385	27	188	215	12.558	29	174	203	14.286
	3	22	157	179	12.291	25	157	182	13.736				
	4	18	146	164	10.976	18	146	164	10.976	16	158	174	9.195
	5	23	163	186	12.366	23	165	188	12.234				
	6	28	207	235	11.915	27	211	238	11.345	31	197	228	13.596
	7	24	186	210	11.429	24	183	207	11.594				
	8	31	153	184	16.848	33	155	188	17.553	29	163	192	15.104
	9	15	152	167	8.982	15	150	165	9.091				
	10	17	170	187	9.091	17	173	190	8.947	16	169	185	8.649
Total		**232**	**1683**	**1915**	**12.115**	**235**	**1693**	**1928**	**12.189**	**121**	**861**	**982**	**12.322**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	6	3	9	66.667	6	3	9	66.667				
TJL88/673	**2**	7	3	10	70.000	7	3	10	70.000	7	2	9	77.778
	3	6	2	8	75.000	6	2	8	75.000				
	4	5	1	6	83.333	5	1	6	83.333	5	1	6	83.333
	5	7	1	8	87.500	7	1	8	87.500				
	6	5	2	7	71.429	5	2	7	71.429	5	2	7	71.429
	7	4	2	6	66.667	4	2	6	66.667				
	8	8	0	8	100.000	8	0	8	100.000	8	0	8	100
	9	5	1	6	83.333	5	1	6	83.333				
	10	5	1	6	83.333	5	1	6	83.333	5	2	7	71.429
Total		**58**	**16**	**74**	**78.378**	**58**	**16**	**74**	**78.378**	**30**	**7**	**37**	**81.081**

8.1.2.2 Tfe3 Immunoreactivity in CGCG

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	4	144	148	2.703	4	144	148	2.703				
TJL91/651	**2**	4	148	152	2.632	4	144	148	2.703				
	3	13	232	245	5.306	14	223	237	5.907				
	4	2	175	177	1.130	2	167	169	1.183				
	5	1	143	144	0.694	1	143	144	0.694				
	6	8	157	165	4.848	8	157	165	4.848				
	7	4	127	131	3.053	5	124	129	3.876				
	8	3	220	223	1.345	4	223	227	1.762				
	9	3	136	139	2.158	3	140	143	2.098				
	10	9	165	174	5.172	4	176	180	2.222				
Total		**51**	**1647**	**1698**	**3.004**	**49**	**1641**	**1690**	**2.899**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	2	0	2	100.000	2	0	2	100.000				
TJL91/651	**2**	0	3	3	0.000	0	3	3	0.000				
	3	2	1	3	66.667	2	1	3	66.667				
	4	1	3	4	25.000	1	3	4	25.000				
	5	0	3	3	0.000	0	3	3	0.000				
	6	2	1	3	66.667	2	1	3	66.667				
	7	1	2	3	33.333	1	2	3	33.333				
	8	0	1	1	0.000	0	1	1	0.000				
	9	4	0	4	100.000	4	0	4	100.000				
	10	3	1	4	75.00	3	1	4	75.00				
Total		**15**	**15**	**30**	**50.000**	**15**	**15**	**30**	**50.000**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	5	139	144	3.472	5	137	142	3.521				
TJL94/271	**2**	5	156	161	3.106	5	156	161	3.106	5	163	168	2.976
	3	6	126	132	4.545	6	126	136	4.545				
	4	6	110	116	5.172	6	110	116	5.172	7	105	112	6.250
	5	7	166	173	4.046	7	166	173	4.046				
	6	4	163	167	2.395	4	160	164	2.439	5	159	164	3.049
	7	2	169	171	1.170	2	166	168	1.190				
	8	2	186	188	1.064	2	182	184	1.087	2	187	189	1.058
	9	3	195	198	1.515	3	198	201	1.493				
	10	1	204	205	0.488	1	204	205	0.488	1	203	204	0.490
Total		**41**	**1614**	**1655**	**2.477**	**41**	**1605**	**1646**	**2.491**	**20**	**817**	**837**	**2.389**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	6	6	12	50.000	6	6	12	50.000				
TJL94/271	**2**	7	4	11	63.636	7	4	11	63.636	7	3	10	70.000
	3	9	2	11	81.818	9	2	11	81.818				
	4	5	5	10	50.000	5	5	10	50.000	5	6	11	45.454
	5	5	7	12	41.667	5	7	12	41.667				
	6	4	5	9	44.444	4	5	9	44.444	6	4	10	60.000
	7	3	5	8	37.500	3	5	8	37.500				
	8	3	7	10	30.000	3	7	10	30.000	2	8	10	20.000
	9	2	4	6	33.333	2	4	6	33.333				
	10	2	6	8	25.000	2	6	8	25.000	2	6	8	25.000
Total		**46**	**51**	**97**	**47.423**	**46**	**51**	**97**	**47.423**	**22**	**27**	**49**	**44.898**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	12	145	157	7.643	12	145	157	7.643				
TJ1018195	**2**	15	94	109	13.761	15	97	112	13.393	13	84	97	13.402
	3	14	113	127	11.024	14	113	127	11.024				
	4	34	107	141	24.113	36	105	141	25.532	29	103	132	21.970
	5	15	146	161	9.317	15	146	161	9.317				
	6	13	84	97	13.402	13	84	97	13.402	11	95	106	10.377
	7	13	134	147	8.844	13	136	149	8.725				
	8	15	108	123	12.195	15	108	123	12.195	21	95	116	18.103
	9	16	155	171	9.357	16	157	173	9.249				
	10	14	125	139	10.072	14	125	139	10.072	15	145	160	9.375
Total		**70**	**1184**	**1345**	**11.970**	**70**	**1216**	**1379**	**11.820**	**89**	**522**	**611**	**14.567**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	2	3	5	40.000	2	3	5	40.000				
TJA1018195	**2**	5	0	5	100.000	5	0	5	100.000	5	0	5	100.000
	3	5	3	8	62.500	5	3	8	62.500				
	4	6	0	6	100.000	6	0	6	100.000	7	0	7	100.000
	5	6	4	10	60.000	6	4	10	60.000				
	6	5	1	6	83.333	5	1	6	83.333	5	1	6	83.333
	7	5	5	10	50.000	5	5	10	50.000				
	8	8	1	9	88.889	8	1	9	88.889	8	1	9	88.889
	9	5	4	9	55.556	5	4	9	55.556				
	10	2	2	4	50.000	2	2	4	50.000	3	1	4	75.000
Total		**49**	**23**	**72**	**68.056**	**49**	**23**	**72**	**68.056**	**28**	**3**	**31**	**90.323**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	14	141	155	9.032	12	150	162	7.407				
TJL97/491	**2**	15	176	191	7.853	14	188	202	6.931				
	3	9	226	235	3.830	8	214	222	3.604				
	4	11	168	179	6.145	9	162	171	5.263				
	5	8	176	184	4.348	8	176	184	4.348				
	6	7	184	191	3.665	7	185	192	3.646				
	7	13	160	173	7.514	15	163	178	8.427				
	8	7	192	199	3.518	9	200	209	4.306				
	9	3	218	221	1.357	3	218	221	1.357				
	10	4	149	153	2.614	3	140	143	2.143				
Total		**91**	**1790**	**1881**	**4.838**	**88**	**1796**	**1884**	**4.671**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	6	0	6	100.000	6	0	6	100.000				
TJL97/491	**2**	5	1	6	83.333	5	1	6	83.333				
	3	1	4	5	20.000	1	4	5	20.000				
	4	2	2	4	50.000	2	2	4	50.000				
	5	3	3	6	50.000	3	3	6	50.000				
	6	1	2	3	33.333	1	2	3	33.333				
	7	0	5	5	0.000	0	5	5	0.000				
	8	1	2	3	33.333	1	2	3	33.333				
	9	0	3	3	0.000	0	3	3	0.000				
	10	2	2	4	50.000	2	2	4	50.000				
Total		**21**	**24**	**45**	**46.667**	**21**	**24**	**45**	**46.667**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	10	244	254	3.937	10	250	260	3.846				
TJL98/370	**2**	7	162	169	4.142	7	162	169	4.142	6	147	153	3.922
	3	2	244	246	0.813	2	243	245	0.816				
	4	9	158	167	5.389	9	158	167	5.389	10	147	157	6.369
	5	5	187	192	2.604	5	192	197	2.538				
	6	20	164	184	10.870	19	166	185	10.270	21	167	188	11.170
	7	12	197	209	5.742	12	192	204	5.882				
	8	9	188	197	4.569	9	186	195	4.615	9	209	218	4.128
	9	4	189	193	2.073	4	193	197	2.030				
	10	5	202	207	2.415	5	201	206	2.427	4	202	206	1.942
Total		**83**	**1935**	**2018**	**4.113**	**82**	**1943**	**2025**	**4.049**	**50**	**872**	**722**	**5.423**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	0	9	9	0.000	0	9	9	0.000				
TJL98/370	**2**	4	11	15	26.667	4	11	15	26.667	5	12	17	29.412
	3	1	7	8	12.500	1	7	8	12.500				
	4	5	8	13	38.462	5	8	13	38.462	7	5	10	70.000
	5	2	11	13	15.385	2	11	13	15.385				
	6	6	1	7	85.714	6	1	7	85.714	7	1	8	87.500
	7	4	7	11	36.364	4	7	11	36.364				
	8	1	5	6	16.667	1	5	6	16.667	1	5	6	46.667
	9	2	8	10	20.000	2	8	10	20.000				
	10	1	5	6	16.667	1	5	6	16.667	1	5	6	16.667
Total		**26**	**72**	**98**	**26.531**	**26**	**72**	**98**	**26.531**	**21**	**26**	**47**	**44.681**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	21	169	190	11.053	21	170	191	10.995				
TJL02/068	**2**	19	173	192	9.896	19	173	192	9.896				
	3	15	169	184	8.152	15	172	187	8.021				
	4	13	140	153	8.497	14	135	149	9.396				
	5	17	118	135	12.593	17	118	135	12.593				
	6	8	106	114	7.018	8	106	114	7.018				
	7	8	116	124	6.452	9	115	124	7.258				
	8	14	132	146	9.589	14	134	148	9.459				
	9	14	99	113	12.390	14	102	116	12.069				
	10	18	145	163	11.043	15	149	164	9.146				
Total		**147**	**1367**	**1514**	**9.709**	**146**	**1374**	**1520**	**9.605**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
6	**1**	1	7	8	12.500	1	7	8	12.500				
TJL02/068	**2**	1	6	7	14.286	1	6	7	14.286				
	3	0	7	7	0.000	0	7	7	0.000				
	4	2	6	8	25.000	2	6	8	25.000				
	5	2	3	5	40.000	2	3	5	40.000				
	6	0	5	5	0.000	0	5	5	0.000				
	7	0	5	5	0.000	0	5	5	0.000				
	8	0	4	4	0.000	0	4	4	0.000				
	9	1	2	3	33.333	1	2	3	33.333				
	10	3	3	5	50.000	3	3	5	50.000				
Total		**10**	**48**	**58**	**17.241**	**10**	**48**	**58**	**17.241**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	11	100	111	9.910	11	102	113	9.735				
TJL91/581	**2**	13	93	106	12.264	13	93	106	12.264	15	111	126	11.975
	3	9	123	132	6.818	9	124	133	6.767				
	4	14	50	64	21.875	14	49	63	22.222	16	51	67	23.881
	5	8	116	124	6.452	8	113	121	6.612				
	6	22	86	108	20.370	22	86	108	20.370	23	83	106	21.698
	7	6	81	87	6.897	6	81	87	6.897				
	8	38	90	128	29.688	38	88	126	30.159	43	81	124	34.677
	9	11	84	95	11.579	11	84	95	11.579				
	10	32	102	134	23.881	32	106	138	23.188	30	94	124	24.194
Total		**164**	**925**	**1089**	**15.060**	**164**	**926**	**1090**	**15.046**	**127**	**420**	**547**	**23.218**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
7	**1**	0	**7**	**7**	0.000	0	**7**	**7**	0.000				
TJL91/581	**2**	0	5	5	0.000	0	5	5	0.000	0	6	6	0
	3	0	4	4	0.000	0	4	4	0.000				
	4	1	7	8	12.500	1	7	8	12.500	1	6	7	14.286
	5	0	5	5	0.000	0	5	5	0.000				
	6	2	9	11	18.182	2	9	11	18.182	3	8	11	27.273
	7	1	7	8	12.500	1	7	8	12.500				
	8	1	6	7	14.286	1	6	7	14.286	1	6	7	14.286
	9	0	4	4	0.000	0	4	4	0.000				
	10	2	8	10	20.000	2	8	10	20.000	3	5	8	37.500
Total		**7**	**62**	**69**	**10.145**	**7**	**62**	**69**	**10.145**	**8**	**31**	**39**	**20.513**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	4	149	153	2.614	4	150	154	2.597				
TJL90/409	**2**	2	151	153	1.307	2	151	153	1.307				
	3	6	215	221	2.715	6	219	225	2.667				
	4	7	186	193	3.627	7	183	190	3.684				
	5	3	172	175	1.714	3	172	175	1.714				
	6	4	152	156	2.564	4	148	152	2.632				
	7	2	153	155	1.290	2	153	155	1.290				
	8	3	176	179	1.676	3	180	183	1.639				
	9	3	121	124	2.419	2	117	119	1.681				
	10	3	174	177	1.695	3	169	172	1.744				
Total		**37**	**1649**	**1686**	**2.195**	**36**	**1642**	**1678**	**2.145**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
8	**1**	3	4	7	42.857	3	4	7	42.857				
TJL90/409	**2**	3	5	8	37.500	3	5	8	37.500				
	3	0	5	5	0.000	0	5	5	0.000				
	4	1	6	7	14.286	1	6	7	14.286				
	5	1	3	4	25.000	1	3	4	25.000				
	6	1	5	6	16.667	1	5	6	16.667				
	7	0	8	8	0.000	0	8	8	0.000				
	8	1	6	7	14.286	1	6	7	14.286				
	9	2	8	10	20.000	2	8	10	20.000				
	10	2	7	9	22.222	2	7	9	22.222				
Total		**14**	**57**	**71**	**19.718**	**14**	**57**	**71**	**19.718**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	11	107	118	9.322	11	107	118	9.322				
TJL90/075	**2**	10	69	79	12.658	10	70	80	12.500	5	89	94	5.319
	3	7	95	102	6.863	7	95	102	6.863				
	4	9	89	98	9.184	9	89	98	9.184	11	76	87	12.644
	5	2	81	83	2.410	2	81	83	2.410				
	6	9	54	63	14.286	9	56	65	13.846	9	49	58	15.517
	7	11	115	126	8.730	10	117	127	7.874				
	8	14	65	79	17.722	15	61	76	19.737	20	58	78	25.641
	9	4	73	77	5.195	4	73	77	5.195				
	10	10	73	83	12.048	11	72	83	13.253	14	67	81	17.284
Total		**87**	**821**	**908**	**9.581**	**88**	**821**	**909**	**9.681**	**59**	**339**	**398**	**14.824**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
9	**1**	2	3	5	40.000	2	3	5	40.000				
TJL90/075	**2**	1	1	2	50.000	1	1	2	50.000	1	0	1	100.000
	3	1	2	3	33.333	1	2	3	33.333				
	4	7	0	7	100.000	7	0	7	100.000	7	0	7	100.000
	5	5	1	6	83.333	5	1	6	83.333				
	6	3	0	3	100.000	3	0	3	100.000	3	0	3	100.000
	7	1	1	2	50.000	1	1	2	50.000				
	8	2	0	2	100.000	2	0	2	100.000	2	0	2	100.000
	9	2	1	3	66.667	2	1	3	66.667				
	10	4	2	6	66.667	4	2	6	66.667	4	1	5	80.000
Total		**28**	**11**	**39**	**71.795**	**28**	**11**	**39**	**71.795**	**17**	**1**	**18**	**94.444**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	2	121	123	1.626	2	121	123	1.626				
TJL89/488	**2**	1	138	139	0.719	1	140	141	0.709				
	3	7	96	103	6.863	6	97	103	5.825				
	4	4	69	73	5.479	4	69	73	5.479				
	5	5	99	104	4.808	5	99	104	4.808				
	6	2	126	128	1.563	2	125	127	1.575				
	7	8	121	129	6.202	8	124	132	6.061				
	8	2	105	107	1.869	2	105	107	1.869				
	9	1	120	121	0.826	1	120	121	0.826				
	10	0	117	117	0.000	0	117	117	0.000				
Total		**32**	**1112**	**1144**	**2.797**	**31**	**1117**	**1148**	**2.700**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
10	**1**	0	7	7	0.000	0	7	7	0.000				
TJL89/488	**2**	0	3	3	0.000	0	3	3	0.000				
	3	2	4	6	33.333	2	4	6	33.333				
	4	1	3	4	25.000	1	3	4	25.000				
	5	0	3	3	0.000	0	3	3	0.000				
	6	0	6	6	0.000	0	6	6	0.000				
	7	2	7	9	22.222	2	7	9	22.222				
	8	1	3	4	25.000	1	3	4	25.000				
	9	0	5	5	0.000	0	5	5	0.000				
	10	0	3	3	0.000	0	3	3	0.000				
Total		**6**	**44**	**50**	**12.000**	**6**	**44**	**50**	**12.000**				

8.1.2.3 Tfe3 Immunoreactivity in cherubism

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	6	101	107	5.607	6	101	107	5.607				
TJL03/887	**2**	9	119	128	7.031	9	121	130	6.923	10	111	121	8.264
	3	8	109	117	6.838	8	107	115	6.957				
	4	7	128	135	5.185	7	128	135	5.185	9	134	143	6.294
	5	7	154	161	4.348	7	156	163	4.294				
	6	15	123	138	10.870	15	124	139	10.791	17	117	134	12.687
	7	5	142	147	3.401	5	145	150	3.333				
	8	6	108	114	5.263	6	106	112	5.357	4	112	116	3.448
	9	1	101	102	0.980	1	101	102	0.980				
	10	3	120	123	2.439	3	121	124	2.419	1	119	120	0.833
Total		**67**	**1205**	**1272**	**5.627**	**67**	**1210**	**1277**	**5.247**	**41**	**593**	**634**	**6.467**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	**1**	5	3	8	62.500	5	3	8	62.500				
TJL03/887	**2**	5	3	8	62.500	5	3	8	62.500	5	4	9	55.556
	3	1	8	9	11.111	1	8	9	11.111				
	4	1	3	4	25.000	1	3	4	25.000	1	2	3	33.333
	5	3	4	7	42.857	3	4	7	42.857				
	6	1	5	6	16.667	1	5	6	16.667	1	5	6	16.667
	7	2	9	11	18.182	2	9	11	18.182				
	8	2	10	12	16.667	2	10	12	16.667	2	10	12	16.667
	9	1	6	7	14.286	1	6	7	14.286				
	10	1	5	6	16.667	1	5	6	16.667	1	5	6	16.667
Total		**22**	**56**	**78**	**28.205**	**22**	**56**	**78**	**28.205**	**10**	**26**	**36**	**27.778**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	1	143	144	0.694	1	143	144	0.694				
TJL95/763	**2**	1	102	103	0.971	1	100	101	0.991				
	3	0	160	160	0.000	0	163	163	0.000				
	4	5	107	112	4.464	4	110	114	3.509				
	5	0	111	111	0.000	0	113	113	0.000				
	6	2	128	130	1.538	2	133	135	1.481				
	7	7	127	134	5.224	7	132	139	5.036				
	8	5	115	120	4.167	5	117	122	4.098				
	9	10	121	131	7.634	9	120	129	6.977				
	10	7	85	92	7.609	7	85	92	7.609				
Total		**38**	**1199**	**1237**	**3.072**	**36**	**1216**	**1252**	**2.875**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	0	3	3	0.000	0	3	3	0.000				
TJL95/763	**2**	0	4	4	0.000	0	4	4	0.000				
	3	0	5	5	0.000	0	5	5	0.000				
	4	0	4	4	0.000	0	4	4	0.000				
	5	0	6	6	0.000	0	6	6	0.000				
	6	0	3	3	0.000	0	3	3	0.000				
	7	1	1	2	50.000	1	1	2	50.000				
	8	2	4	6	33.333	2	4	6	33.333				
	9	1	5	6	16.667	1	5	6	16.667				
	10	2	4	6	33.333	2	4	6	33.333				
Total		**6**	**39**	**45**	**13.333**	**6**	**39**	**45**	**13.333**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	14	102	116	12.069	14	102	116	12.069				
TJL96/188	**2**	12	128	140	8.571	12	130	142	8.451				
	3	8	105	113	7.080	8	105	113	7.080				
	4	11	75	86	12.791	11	75	86	12.791				
	5	19	98	117	16.239	17	98	115	14.783				
	6	21	85	106	19.811	21	85	106	19.811				
	7	10	99	109	9.174	10	98	108	9.259				
	8	20	96	116	17.241	20	96	116	17.241				
	9	23	190	213	10.798	22	192	214	10.280				
	10	16	98	114	14.035	16	99	115	13.913				
Total		**154**	**1076**	**1230**	**12.520**	**151**	**1080**	**1231**	**12.266**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	1	1	2	50.000	1	1	2	50.000				
TJL96/188	**2**	1	2	3	33.333	1	2	3	33.333				
	3	1	2	3	33.333	1	2	3	33.333				
	4	1	3	4	25.000	1	3	4	25.000				
	5	2	3	5	40.000	2	3	5	40.000				
	6	2	1	3	66.667	2	1	3	66.667				
	7	2	3	5	40.000	2	3	5	40.000				
	8	5	2	7	71.429	5	2	7	71.429				
	9	3	4	7	42.857	3	4	7	42.857				
	10	2	3	5	40.000	2	3	5	40.000				
Total		**20**	**24**	**44**	**45.455**	**20**	**24**	**44**	**45.455**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	4	93	97	4.124	4	93	97	4.124				
TJL99/665	**2**	12	67	79	15.190	12	67	79	15.190	11	71	82	13.415
	3	9	105	114	7.895	9	105	114	7.895				
	4	9	65	74	12.162	9	64	73	12.329	13	61	74	17.568
	5	20	107	127	15.748	20	105	125	13.158				
	6	19	96	115	16.522	19	96	115	16.522	19	89	108	17.593
	7	4	97	101	3.960	4	97	101	3.960				
	8	15	102	117	12.821	15	102	117	12.821	10	111	121	8.265
	9	6	84	90	6.667	6	84	90	6.667				
	10	8	88	96	8.333	8	88	96	8.333	8	84	92	8.696
Total		**106**	**904**	**1010**	**10.495**	**106**	**901**	**1007**	**10.526**	**61**	**416**	**477**	**12.789**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	2	3	5	40.000	2	3	5	40.000				
TJL99/665	**2**	1	1	2	50.000	1	1	2	50.000	1	1	2	50.000
	3	6	3	9	66.667	6	3	9	66.667				
	4	4	0	4	100.000	4	0	4	100.000	4	0	4	100.000
	5	2	4	6	33.333	2	4	6	33.333				
	6	1	0	1	100.000	1	0	1	100.000	1	0	1	100.000
	7	2	4	6	33.333	2	4	6	33.333				
	8	3	0	3	100.000	3	0	3	100.000	3	0	3	100.000
	9	2	3	5	40.000	2	3	5	40.000				
	10	2	1	3	66.667	2	1	3	66.667	2	1	3	66.667
Total		**25**	**19**	**44**	**56.818**	**25**	**19**	**44**	**56.818**	**11**	**2**	**13**	**84.615**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	8	126	134	5.970	8	127	135	5.926				
TJL99/223	**2**	13	125	138	9.420	13	125	138	9.420	7	141	148	4.730
	3	4	114	118	3.390	3	115	118	2.542				
	4	4	138	142	2.817	4	138	142	2.817	5	132	137	3.650
	5	3	110	113	2.655	3	110	113	2.655				
	6	17	95	112	15.179	17	97	114	14.912	17	92	109	15.596
	7	5	140	145	3.448	5	138	143	3.497				
	8	34	135	169	20.118	35	132	167	20.958	39	135	174	22.414
	9	14	133	147	9.524	12	135	147	8.163				
	10	9	121	130	6.923	9	121	130	6.923	9	113	122	7.377
Total		**111**	**1237**	**1348**	**8.234**	**109**	**1238**	**1347**	**8.092**	**77**	**613**	**690**	**11.159**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	4	2	6	66.667	4	2	6	66.667				
TJL99/223	**2**	10	1	11	90.909	10	1	11	90.909	12	1	13	92.310
	3	8	1	9	88.889	8	1	9	88.889				
	4	10	2	12	83.333	10	2	12	83.333	9	2	11	81.818
	5	6	7	13	46.154	6	7	13	46.154				
	6	8	2	10	80.000	8	2	10	80.000	7	3	10	70.000
	7	6	3	9	66.667	6	3	9	66.667				
	8	10	1	11	90.909	10	1	11	90.909	9	1	10	90.000
	9	5	7	12	41.667	5	7	12	41.667				
	10	8	3	11	72.727	8	3	11	72.727	8	4	12	66.667
Total		**75**	**29**	**104**	**72.115**	**75**	**29**	**104**	**72.115**	**45**	**11**	**56**	**80.357**

8.1.2.4 Tfe3 Immunoreactivity in ABC

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	1	2	124	126	1.587	2	124	126	1.587				
TJL88/279	2	10	160	170	5.882	11	164	175	6.286	11	178	189	5.820
	3	3	165	168	1.786	3	164	167	1.796				
	4	14	165	179	7.821	14	165	179	7.821	13	183	196	6.633
	5	3	152	155	1.935	3	152	155	1.935				
	6	9	179	188	4.787	9	178	187	4.813	10	155	165	6.061
	7	2	107	109	1.835	2	106	108	1.852				
	8	19	186	205	9.268	19	186	205	9.268	19	163	182	10.440
	9	2	127	129	1.550	2	127	129	1.550				
	10	10	178	188	5.319	12	177	189	6.349	11	175	186	5.914
Total		**74**	**1543**	**1617**	**4.576**	**77**	**1543**	**1620**	**4.753**	**64**	**854**	**918**	**6.972**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
1	1	0	3	3	0.000	0	3	3	0.000				
TJL88/279	2	0	0	0	0.000	0	0	0	0.000	0	0	0	0.000
	3	0	4	4	0.000	0	4	4	0.000				
	4	0	2	2	0.000	0	2	2	0.000	0	2	2	0.000
	5	1	4	5	20.000	1	4	5	20.000				
	6	0	0	0	0.000	0	0	0	0.000	0	0	0	0.000
	7	2	3	5	40.000	2	3	5	40.000				
	8	0	2	2	0.000	0	2	2	0.000	0	2	2	0.000
	9	0	4	4	0.000	0	4	4	0.000				
	10	0	0	2	0.000	0	0	2	0.000	0	1	1	0.000
Total		**3**	**24**	**27**	**11.111**	**3**	**24**	**27**	**11.111**	**0**	**5**	**5**	**0.000**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	1	60	18	78	76.923	58	20	78	74.359				
TJL88/593	2	62	25	87	71.264	59	26	85	69.412	65	24	89	73.034
	3	69	24	93	74.194	67	25	92	72.826				
	4	47	23	70	67.143	41	23	64	64.063	56	22	78	71.795
	5	53	41	94	56.388	53	41	94	56.383				
	6	69	31	100	69.000	68	31	99	68.687	73	29	102	71.569
	7	61	21	82	74.390	61	21	82	74.390				
	8	77	33	110	70.000	76	37	113	67.257	74	33	107	69.159
	9	41	38	79	51.899	41	37	78	52.564				
	10	46	26	72	63.889	45	27	72	62.500	47	22	69	68.116
Total		**585**	**280**	**865**	**67.630**	**569**	**288**	**857**	**66.394**	**315**	**130**	**445**	**70.787**

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
2	**1**	3	4	7	42.857	3	4	7	42.857				
TJL88/593	**2**	3	6	9	33.333	3	6	9	33.333	3	7	10	30.000
	3	3	1	4	75.000	3	1	4	75.000				
	4	3	0	3	100.000	3	0	3	100.000	3	0	3	100.000
	5	3	1	4	75.000	3	1	4	75.000				
	6	0	2	2	0.000	0	2	2	0.000	0	2	2	0.000
	7	2	1	3	66.667	2	1	3	66.667				
	8	1	1	2	50.000	1	1	2	50.000	1	1	2	50.000
	9	3	1	4	75.000	3	1	4	75.000				
	10	3	1	4	75.000	3	1	4	75.000	3	1	4	75.000
Total		**24**	**18**	**42**	**57.143**	**24**	**18**	**42**	**57.143**	**10**	**11**	**21**	**47.619**

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	20	145	165	12.121	17	155	172	9.884				
TJL99/489	**2**	16	151	167	9.581	16	153	169	9.468				
	3	20	119	139	14.389	19	127	146	13.014				
	4	19	128	147	12.925	20	132	152	13.158				
	5	15	104	119	12.605	15	106	121	12.397				
	6	11	123	134	8.290	10	119	129	7.752				
	7	14	112	126	11.111	14	108	122	11.475				
	8	15	139	154	9.740	13	136	149	8.725				
	9	13	163	176	7.386	13	169	182	7.143				
	10	13	142	155	8.387	14	146	160	8.750				
Total		**156**	**1326**	**1482**	**10.526**	**151**	**1351**	**1502**	**10.053**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
3	**1**	1	4	5	20.000	1	4	5	20.000				
TJL99/489	**2**	0	3	3	0.000	0	3	3	0.000				
	3	2	3	5	40.000	2	3	5	40.000				
	4	3	2	5	60.000	3	2	5	60.000				
	5	3	0	3	100.000	3	0	3	100.000				
	6	1	2	3	33.333	1	2	3	33.333				
	7	3	3	6	50.000	3	3	6	50.000				
	8	5	3	8	62.500	5	3	8	62.500				
	9	1	3	4	25.000	1	3	4	25.000				
	10	5	4	9	55.556	5	4	9	55.556				
Total		**24**	**27**	**51**	**47.059**	**24**	**27**	**51**	**47.059**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	20	98	118	16.949	20	99	119	16.807				
TJA0937588	**2**	11	73	84	13.095	11	75	86	12.791				
	3	22	70	92	23.913	20	72	92	21.739				
	4	23	116	139	16.547	23	114	137	16.788				
	5	32	94	126	25.397	31	90	121	25.620				
	6	32	111	143	22.378	32	112	144	22.222				
	7	33	102	135	24.444	33	102	135	24.444				
	8	32	100	132	24.242	32	102	134	23.881				
	9	13	119	132	9.848	13	117	130	10.000				
	10	26	95	131	21.488	26	95	121	21.488				
Total		**244**	**978**	**1222**	**19.967**	**241**	**978**	**1219**	**19.770**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
4	**1**	4	0	4	100.000	4	0	4	100.000				
TJA0937588	**2**	4	1	5	80.000	4	1	5	80.000				
	3	4	0	4	100.000	4	0	4	100.000				
	4	9	0	9	100.000	9	0	9	100.000				
	5	8	0	8	100.000	8	0	8	100.000				
	6	4	2	6	66.667	4	2	6	66.667				
	7	5	0	5	100.000	5	0	5	100.000				
	8	3	0	3	100.000	3	0	3	100.000				
	9	3	0	3	100.000	3	0	3	100.000				
	10	5	0	5	100.000	5	0	5	100.000				
Total		**49**	**3**	**52**	**94.231**	**49**	**3**	**52**	**94.231**				

Case No.	Field No.	STROMAL CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	3	138	141	2.128	3	141	144	2.083				
TJL87/725	**2**	2	114	116	1.724	2	116	118	1.695				
	3	4	145	149	2.685	4	145	149	2.685				
	4	5	133	138	3.623	5	133	138	3.623				
	5	6	119	125	4.800	6	119	125	4.800				
	6	2	60	62	3.333	2	60	62	3.333				
	7	3	54	57	5.263	3	54	57	5.263				
	8	2	96	98	2.041	2	96	98	2.041				
	9	2	114	116	1.724	2	114	116	1.724				
	10	3	118	121	2.480	3	118	121	2.480				
Total		**32**	**1091**	**1123**	**2.850**	**32**	**1096**	**1128**	**2.837**				

Case No.	Field No.	GIANT CELLS											
		INTRA-OBSERVER								INTER-OBSERVER			
		1ST Count				2ND Count							
		+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index	+ve	-ve	Total	Labelling Index
5	**1**	2	2	4	50.000	2	2	4	50.000				
TJL87/725	**2**	1	1	2	50.000	1	1	2	50.000				
	3	3	1	4	75.000	3	1	4	75.000				
	4	2	2	4	50.000	2	2	4	50.000				
	5	2	3	5	40.000	2	3	5	40.000				
	6	2	1	3	66.667	2	1	3	66.667				
	7	1	1	2	50.000	1	1	2	50.000				
	8	1	0	1	100.000	1	0	1	100.000				
	9	2	0	2	100.000	2	0	2	100.000				
	10	3	0	3	100.000	3	0	3	100.000				
Total		**19**	**11**	**30**	**63.333**	**19**	**11**	**30**	**63.333**				

Annexure B – Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Altini

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080850**
PROJECT	Approval for Research Conducted on Archived Paraffin Wax Embedded Tissue Blocks (Original Class Approval M00/08/29)
INVESTIGATORS	Prof M Altini
DEPARTMENT	Dept of Oral Pathology
DATE CONSIDERED	08.09.10
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 23.09.08 **CHAIRPERSON** .....
pp (Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof M Altini

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

CHAPTER 9

9.0 REFERENCES

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