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CLINICOPATHOLOGICAL EVALUATION OF FOCAL REACTIVE LESIONS OF THE GINGIVA

Cliff Mirirai Karuma

A Research report submitted to the Faculty of Health Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry.

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Plagiarism declaration

I Cliff Mirirai Karuma declare that this Research Report is my own, unaided work. It is being submitted for the Degree of MSc (Dentistry) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

15th day of May 2020 in Zimbabwe

Dedication

This research report is dedicated to my lovely wife Charity, whose affection and support was instrumental in the completion of this work.

Abstract

Introduction: Focal reactive gingival lesions are elicited by chronic irritation primarily due to poor oral hygiene, including plaque accumulation, calculus, over-hanging dental restorations and ill-fitting dental prosthesis among others. Persistent irritation of the gingiva can lead to tissue injury and trigger inflammation leading to proliferation of endothelial cells, multi-nucleated giant cells, fibroblasts and tissue mineralisation. The aim of the study was to report the clinicopathological features of focal reactive gingival lesions.

Methods: Utilising convenient sampling, records were retrieved from the Department of Oral Pathology and the Department of Oral Medicine and Periodontology at the Wits Oral Health Centre, to perform a retrospective cross-sectional study. Sociodemographic variables (age, sex and occurrence of lesion during pregnancy) and clinical features (pedunculated/sessile lesion, size of lesion, colour, region of jaw affected) were recorded.

Results: Female patients accounted for 70.8% (n = 172) of all focal reactive gingival lesions, whereas the male patients accounted for 29.2% (n = 71). A total of 56.4% (n = 137) of the lesions occurred on the maxilla compared to 43.6% (n = 106) on the mandible. The age of patients (irrespective of diagnosis and sex) ranged from 3 months to 88 years. A total of 60.1% (n = 146) of all lesions were erythematous compared to 39.9% (n = 97) that were non-erythematous.

Conclusion: After analysing 243 cases, contrary to findings in other studies, the peripheral ossifying fibroma was the most common focal reactive gingival lesion.

Acknowledgements

This study would not have been possible without the immense help and guidance of my supervisors Dr Sizakele Ngwenya and Professor Sindisiwe Shangase.

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Nomenclature

FRGL : Focal reactive gingival lesions

PG : Pyogenic granuloma

FFH : Focal fibrous hyperplasia

POF : Peripheral ossifying fibroma

PGCG : Peripheral giant cell granuloma

R_{rb} : Rank biserial correlation coefficient

DMF : Decayed, Missing and Filled

Chapter 1: Literature review

1.1 Background

Non-neoplastic gingival enlargements can be broadly classified into reactive, developmental or drug-induced (Ababneh and Al-Dwairi, 2005). Focal reactive gingival lesions (FRGL) are elicited by chronic irritation primarily due to over-hanging dental restorations, ill-fitting dental prosthesis, plaque or calculus (Zarei et al., 2007). The gingiva bears the brunt of this chronic irritation and thus is the most common intra-oral site for these lesions (Dutra et al., 2018). Persistent irritation of the gingiva can lead to tissue injury and trigger inflammation leading to proliferation of endothelial cells, multi-nucleated giant cells, fibroblasts and tissue mineralisation (Reddy et al., 2012). The proliferation can eventually lead to a localised hyperplasia and an exuberant gingival mass (Kamal et al., 2012).

Even though FRGL have similar aetiological factors, the various lesions have characteristic histopathological features, and are considered by most authors as separate distinct lesions (Peralles et al., 2006). FRGLs are generally classified into peripheral ossifying fibroma (POF), pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG) and focal fibrous hyperplasia (FFH) (Kfir et al., 1980). Clinically the lesions are similar and histological analysis is required to distinguish these various FRGL (Peralles et al., 2006; Reddy et al., 2012). Some authors dispute that these are distinct lesions and have postulated that these pathological entities represent one lesion at various histological developmental stages (Effiom et al., 2011). They argue that a PG may develop into a POF or FFH with time through further collagen build up and maturation (Prasad et al., 2008). The identical gingival anatomic location of these FRGL adds further weight that these reactive lesions simply represent one lesion at various histopathological stages of development (Hunasgi et al., 2017). However, if this hypothesis is true, a definite age grouping for the different histological entities should be apparent.

The classification and nomenclature by Kfir et al. (1980) has not been adopted by all authors, thus resulting in studies that are slightly confusing to interpret (Tamarit-Borras et al., 2005).

The nomenclature of FRGL is vast and complicated, with various terms used interchangeably to describe the various histopathological features of these FRGL (Kumar et al., 2006; Rossmann 2011). This is best demonstrated where the terms calcifying fibroblastic granuloma, peripheral ossifying fibroma, peripheral cemento-ossifying fibroma, peripheral fibroma with calcification and ossifying fibrous epulis are used to describe the same entity in different studies (Buchner and Hansen, 1987).

FRGLs are more common in females in their second and third decade of life (Rossmann, 2011). The increased risk in females may highlight the influential role hormones may play in the pathogenesis of these lesions (Kenney et al., 1989; Miller et al., 1990; Reddy et al., 2012). The binding of oestrogen to its receptor may stimulate connective tissue proliferation within the gingiva (Gunham et al., 1998). Oestrogen has also been shown to increase osteoclast and osteoblast activity, resulting in bone formation (Whitaker and Bouqout, 1994).

A predilection for the the anterior regions of the jaws (incisor-canine) compared with posterior areas (premolar-molar) has been reported for FRGL (Tamiolakis et al., 2018). Supragingival calculus that predominantly occurs in the anterior mandibular jaw may result in gingivitis which might partly explain the anterior region predilection (Maturana-Ramirez et al., 2015). The cyclical periods of wetness and dryness in the anterior maxilla associated with mouth breathing has also been implicated in the predilection of FRGL in the anterior regions (Agrawal, 2015). Lesions might be uncommon in older individuals because they are often edentulous and rarely seek treatment for painless lesions (Ababneh, 2006). The lesions generally appear as solitary sessile or pedunculated polypoid masses located on the gingiva (Effiom et al., 2011; Franco-Barrera et al., 2015; Shadman et al., 2009). Some authors have reported that PGs are likely to be pedunculated compared to POFs (Peralles et al., 2006). Unless lesions are associated with traumatic ulcers from interference with mastication, pain is not a usual complaint (Bodner and Dayan, 1987).

The average diameter for a PG, FFH and POF is about 20 mm, but the PGCG only averages 10 mm (Effiom et al., 2011). If not surgically resected early PG, POF and FFH can enlarge up to 40 mm in diameter causing difficulties with mastication and disfigurement (Bodner and Dayan, 1987; Chaparro-Avendano et al., 2005; Effiom et al., 2011, Gondivkar, Gadbail, and Chole , 2010). A PGCG rarely grows larger than 20 mm, but lesions larger than 40 mm have

been documented in patients with xerostomia (Bodner et al., 1997; Effiom et al., 2011). The lack of the protective and cleansing action of saliva exacerbates the gingival irritation by calculus or plaque facilitating the growth of the PGCG (Bodner et al., 1997).

Recurrence of FRGL following use of conventional excisional techniques is quite common (Kfir et al., 1980; Salum et al., 2008). Incomplete gingival excision, persistent gingival irritation after excision and removal of lesions by a surgical blade have been reported to increase the risk of recurrence. Deep excisions up to the involved periodontal ligaments, removal of chronic gingival irritants and resection by carbon dioxide lasers have been reported to reduce the recurrence rate (Babu and Hallikeri, 2017). The recurrence rates of POF, PGCG, PG and FFH are 18.5%, 18.2%, 15.6% and 8.9% respectively (Kamal et al., 2012; Katsikeris et al., 1988; Kaustubh et al., 2016; Santos et al., 2014)

1.2 Pyogenic granuloma (PG)

The name PG was coined in 1904 by Hartzell, but it fails to express the true pathological and clinical features of the lesion (Kamal et al., 2012). The name is misleading because no pus or pyogenic material is associated with the gingival mass (Rossmann, 2011). Contamination of the granulation tissue by oral flora in a PG can result in a fibrin exudate, which to the blind eye looks like pus (Kamal et al., 2012). Other names that have been used to describe the PG include granuloma pediculatum, pregnancy tumour, granuloma gravidarum, lobular capillary hemangioma, vascular epulis, Crocker and Hartzell's disease. (Kamal et al., 2012).

Due to the similarity of the histopathological profile of a PG and pregnancy epulis, many authors consider the pregnancy gingival mass a PG (Rossmann, 2011). Sex hormones make the gingiva more susceptible to irritation by calculus and plaque thus enabling a pregnancy epulis to form (Kfir et al., 1980). However, there is no consensus, with other researchers believing the pregnancy associated lesion deserves its own classification based on differences in aetiology, management and pathogenesis (Daley et al., 1991).

Early PG lesions are vascular, reddish in colour and bleed easily on probing (Neville et al., 2009). Older lesions are firmer, pink in colour and more collagenised (Neville et al., 2009;

Prasad et al., 2008). Histologically the lesion is associated with vast areas of capillaries arranged in lobular clusters with a mixed cellular infiltrate of plasma cells, neutrophils and lymphocytes. The surface is lined by stratified squamous epithelium and areas of ulceration may be seen (Neville et al., 2009). Some authors argue that due to this Histopathological picture the term lobular capillary hemangioma is more appropriate terminology (Mills et al., 1980).

Based on over 5200 cases of FRGL in China, USA, northern India and Iran, the PG comprises approximately 18% to 25% of all reactive gingival lesions (Kfir et al., 1980; Naderi et al., 2012; Reddy et al., 2012; Zhang et al., 2007). This is in stark contrast with a study in Nigeria, where the PG was the most common reactive lesion accounting for 57% of all cases (Effiom, et al., 2011). Studies from Sweden, USA, England, Brazil and southern India have reported that PG has a maxillary jaw predilection (Bhaskar and Jacoway, 1966; Buchner et al., 1977; Kfir et al., 1980; Macleod and Soames, 1987; Salum et al., 2008; Shamim et al., 2008). Though a study from Nigeria found no jaw predilection and one from China found a mandibular jaw predilection (Effiom et al., 2011; Zhang et al., 2007).

1.3 Peripheral ossifying fibroma (POF)

The aetiology of POF is unclear, however there are reports that the lesion may possibly arise from the cells of the periodontal ligament (Moon et al., 2006; Ono et al., 2007). A POF may just represent the histopathological end spectrum of a PG of FFH due to further collagenisation and calcific metaplasia (Prasad et al., 2008). POF generally presents as a pink lesion, with some authors reporting only 36% of the lesions as red (Peralles et al., 2006). The general histopathologic pattern is primarily of a cellular connective tissue stroma associated with mineralised components (Mishra et al., 2011; Shetty et al., 2011). Some studies support the theory that undifferentiated mesenchymal cells from the periodontal ligament form mineralized tissue due to chronic gingival irritation (Shetty et al., 2011). Mesenchymal cells have been shown to be clonogenic been capable of versatile differentiation and having exceptional ability to differentiate into bone (Gronthos et al., 2011). Interleukin-1 and tumor necrosis factor are inflammatory cytokines that have been implicated as causal factors in differentiation and cellular proliferation in human mesenchymal cells (Sun et al., 2014).

A key feature of POF is the increased cellularity in the fibroconnective tissue stroma around the mineralised tissue (Moon et al., 2006). Histologically the hyperplastic squamous epithelium may show areas of ulceration. (Shetty et al., 2011). Early lesions of POF usually present with ulceration covered by a fibrinopurulent membrane (Neville et al., 2002), though some authors argue that ulceration is more a function of trauma (Shetty et al., 2011). The mineralised component is highly variable and can consist of either cementum-like material, bone (woven and lamellar) or dystrophic calcifications (Buchner and Hansen, 1987; Zanaida and Brannon, 2001). In its initial stage POF might misdiagnosed as a PG or FFH because the lesion is mostly composed of a cellular fibroblastic tissue with little areas of ossification (Buchner and Hansen, 1987). Though even with early lesions, the cellular connective tissue stroma is so unmistakable that a histological diagnosis can be made with total conviction (Gardner, 1987). Some authors have shown that the average age of patients with POF is lower than those with PG, thus calling into question the theory that POF may evolve from a pre-existing PG (Buchner et al., 2010). A peripheral odontogenic fibroma, a neoplastic mass can also mimic a POF because mineralised components may also be found in its connective tissue stroma. Though islands of odontogenic epithelium found in the peripheral odontogenic fibroma would clearly differentiate it from a POF (Khot et al., 2017).

Dystrophic calcifications are more commonly seen in early growths whereas older lesions tend to be non-ulcerated and demonstrate cementum or bone elements (Neville et al., 2002). As the POF ages the ulceration heals and the dystrophic calcifications evolve into bone or cementum-like calcifications (Moon et al., 2006; Shetty et al., 2011).

It is generally accepted that the POF has a maxillary jaw predilection (Zhang et al., 2007). Though a study in Nigeria reported that POF are equally distributed between the jaws (Effiom et al., 2011), whilst one study from India found all POF exclusively in the mandible (Kashyap et al., 2012). The POF is generally a lesion of young females and teenagers with a peak incidence in the second and third decades of life (Neville et al., 2002). In contrast, a study from China reported a higher average age of 44 years in patients with POF (Zhang et al., 2007).

1.4 Focal fibrous hyperplasia (FFH)

FFH is also referred to as irritation fibroma, traumatic fibroma, fibrous epulis and fibrous nodule (Neville et al., 2002). The lesion usually presents as a pink, nodular, non-ulcerated, painless gingival mass (Buchner et al., 2010). Histologically there are areas of dense nodular fibrous connective tissue covered by an atrophic stratified squamous epithelium (Santos et al., 2014). In studies of over 4800 reactive gingival lesions from China, USA and Israel, it was reported that FFH was the most common reactive gingival lesion (Buchner et al., 2010; Kfir et al., 1980; Zhang et al., 2007). Though a study in Nigeria found FFH to be the third most common reactive gingival lesion (Effiom et al., 2011). Thus, ethnicity might play a large part in the relative frequencies of these lesions.

Most studies in various countries have reported a female predilection for FFH (Buchner et al., 1977; Effiom et al., 2011; Eversole and Rovin, 1972; Kfir et al., 1980; Reddy et al., 2012; Shamim et al., 2008; Zhang et al., 2007). In contrast one study from India reported a male predominance (Kashyap et al., 2012) whilst one study from Sweden reported no gender predilection (Anneroth and Sirgurdson, 1983). There is no consensus on mean age for patients with FFH. While some authors have reported a peak incidence in the fifth decade of life (Buchner et al., 2010), others have reported a peak incidence in the third decade of life (Effiom et al., 2011).

FFH generally shows no jaw predilection (Buchner et al., 2010; Effiom et al., 2011; Kfir et al., 1980). In contrast two studies, one from Sweden and the other from China reported a mandibular jaw predilection (Anneroth and Sirgurdson, 1983; Zhang et al., 2007).

1.5 Peripheral giant cell granuloma (PGCG)

PGCG has been referred to as giant cell epulis and peripheral giant cell reparative granuloma (Neville et al., 2009). The gingival mass usually presents as a purple to dark red lesion (Chaperro-Avendano et al., 2005). The extensive haemorrhage associated with the lesion will result in accumulation of hemosiderin that will impart the bluish hue (Dutra et al., 2018). PGCG originates from the periodontal membrane and periosteum following chronic trauma

or irritation (Shadman, et al., 2009). Microscopically it shows a vascular connective tissue stroma infiltrated by multinucleated osteoclast-like giant cells (Flaitz, 2000).

PGCG was the least common reactive gingival lesion in most studies accounting for generally less than 11% of all lesions (Buchner et al., 1977; Daley et al., 1991; Effiom et al., 2011; Kfir et al., 1980; Zhang et al., 2007). However, a study from Iran reported the PGCG as the most prevalent reactive gingival lesion (Naderi et al., 2012). The lesion is most common in the fourth decade of life (Giansanti and Waldro, 1969) and shows a mandibular jaw predilection (Buchner et al., 1977).

On sex distribution, studies of PGCG have reported mixed results. While some studies reported no sex predilection (Daley et al., 1991; Kfir et al., 1980), others reported either a predilection for females (Anneroth and Sirgurdson, 1983; Eversole and Rovin, 1972; Giansanti and Waldro, 1969) or males (Naderi et al., 2012).

1.6 Rationale for the study

Studies from different nations report confusing and inconsistent data on the relative frequency of FRGL. Additionally, data on age distribution, sex and jaw predilection is inconsistent as well. Geography, ethnic factors and varying nomenclature of lesions seem to play a pivotal role in some of the divergent reports. Moreover, the lack of consensus on the pathogenesis of these lesions may be contributory to the differences amongst various studies. Some authors believe that focal reactive gingival lesions represent separate clinicopathological entities while others argue that these lesions represent a histopathological spectrum of the same lesion. The high recurrence rates, the apparent association between geography and the relative frequency of lesions, and the lack of information on FRGL in South Africa warrants a study to be undertaken in South Africa.

1.7 Aim

The aim of the study was to report the clinicopathological features of FRGL.

1.8 Objectives

The objectives of the study were:

- To determine the relative frequency of the various FRGL.
- To determine the distribution of FRGL according to sex.
- To determine the distribution of FRGL according to age.
- To determine the distribution of FRGL according to anatomic site.

Chapter 2: Methodology

2.1 Sample

Utilising convenient sampling, patient records from the Department of Oral Pathology and the Department of Oral Medicine and Periodontology at the Witwatersrand Oral Health Centre, a retrospective cross-sectional study was performed. Past dental records from the years 2011 to 2017 were evaluated to select those with a histopathological diagnosis of FRGL according to the guidelines of Kfir et al (1980).

2.2 Inclusion and exclusion criteria

Inclusion criterion was all dental records from which at least 70% of the data required for the study could be extracted (Oliveira, et al., 2015). The exclusion criteria for this study were (1) records of patients on anti-convulsant drugs or calcium channel blockers (Ramu and Rodrigues, 2012), (2) cases diagnosed as epulis fissuratum, (3) reactive lesions presenting on sites other than the gingiva and (4) neoplastic conditions.

2.3 Data collection and variables

Histological and clinical history was obtained from the dental records. Sociodemographic variables (age, sex and occurrence of lesion during pregnancy) and clinical features (pedunculated/sessile lesion, size of lesion, colour, region of jaw affected) were recorded. Gingival lesions were anatomically classified into the following regions anterior (incisor-canine region) and posterior (premolar-molar region) according to the guidelines of Mergoni et al., 2015; Pushparaja and Adyanthaya, 2012. If the mass occupied both regions, it was grouped in the location where the bulk of the lesion was found (Mergoni et al., 2015; Pushparaja and Adyanthaya, 2012).

According to the guidelines of Kfir et al (1980), the FRGL were classified into the following:

(1) PG

(2) POF

(3) FFH

(4) PGCG

2.4 Data analysis

Statistical Package for the Social Sciences (SPSS Version 12.5 for Windows) was used for data analysis. The relative frequency of histologically confirmed cases of FRGL was analysed by determining what percentage of the total number of histopathology cases analysed in the study was PG, POF, FFH or PGCG. For sex, base of attachment and anatomical sites, descriptive statistics were used. For the continuous variables such as age and size of lesions, the Shapiro-Wilk test was used to assess whether data followed a normal distribution. Cross-tabulation analysis was used to summarise the categorical data. The chi-square test was used to assess statistically significant differences for categorical variables. Spearman rank correlation coefficient was applied to assess the correlation between age and size of FRGL. Rank biserial correlation coefficient (R_{rb}) was used to analyse the correlation between, (1) size of FRGL and sex, and (2) size of FRGL and jaw affected. Interpretation of correlation coefficients was according to the guidelines of (Zou et al., 2003).

Table 1: Interpretation of correlation coefficient

Correlation coefficient range	Interpretation
0 - 0.1	No relationship
0.1 - 0.3	Low relationship
0.3 - 0.5	Moderate relationship
0.5 - 0.8	Strong relationship
> 0.8	Very strong relationship

The significance level alpha (α) was set at 0.05 for all statistical tests.

2.5 Ethical considerations

An ethical clearance application was made to the Human Research Ethics Committee of the Faculty of Health Sciences at the University of the Witwatersrand with permission granted (M180672) subsequently. Permission to conduct the study was also sought and obtained from the Witwatersrand Oral Health Centre Hospital Health Risk Committee, the Department of Oral Medicine and Periodontology and Department of Oral Pathology. Patient confidentiality was safeguarded by utilising research identification codes instead of patient's names. The file with the key codes was stored separately from the research data, with very limited access to the key codes. All electronic data pertaining to the research was password protected and was not shared.

Chapter 3: Results

A total of 243 cases of FRGL were evaluated and all were included in the study as per selection criterion. POF constituted the highest number of cases (n = 86, 35.4%) of FRGL as seen in Figure 1.

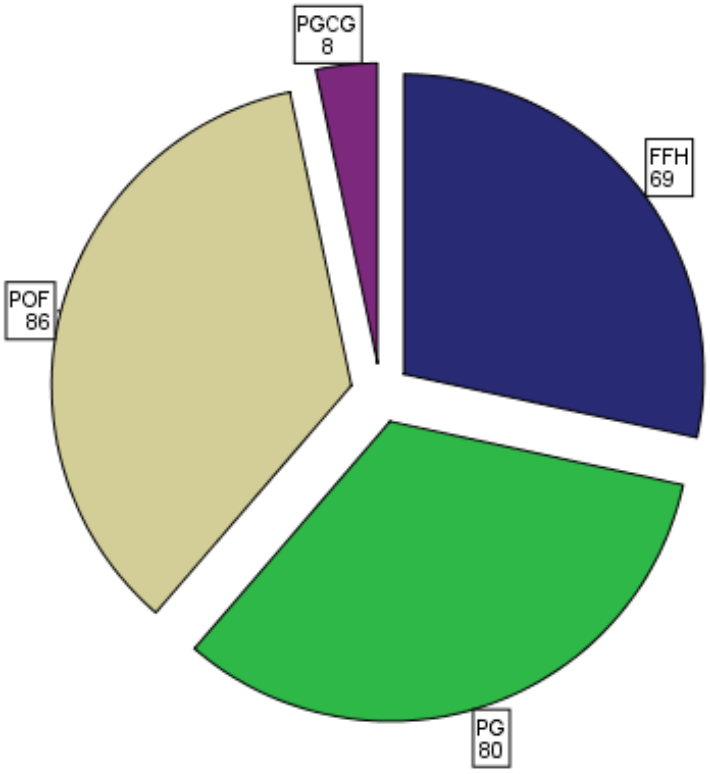

Figure 1: Distribution of FRGL

3.1 Sex

Female patients accounted for 70.8% (n = 172) of all FRGL, whereas the male patients accounted for 29.2% (n = 71) (Table 2).

Table 2: The distribution of FRGL by sex

Diagnosis	Female	Male	Total	P value
FFH	48	21	69	
PG	57	23	80	
POF	62	24	86	0.941
PGCG	5	3	8	
Total	172	71	243	

3.2 Size of FRGL

The size of FRGL ranged from 4mm to 52mm. The smallest was FFH and the largest was the PG (Table 3).

Table 3: Size of FRGL

Lesions	Minimum size (mm)	Maximum size (mm)	Average size (mm)
FFH	4	45	14.65
PG	6	52	17.53
POF	6	35	16.01
PGCG	6	35	16.63

The correlation between size of FRGL and sex was not statistically significant (P = 0.556) as shown in Table 4 below.

Table 4: Correlation between size of FRGL and sex

	Statistical test	Sex	P value
Size	Rank biserial correlation	0.038	0.556

3.3 Age distribution

The age of patients in this cohort ranged from 3 months to 88 years. PGCG had the lowest mean age at presentation (28.4 years), whilst FFH had the highest mean age at presentation (41.8 years) (Table 5). Age did not follow a normal distribution (Shapiro-Wilk test $p > 0.05$).

Table 5: Age distribution

Diagnosis	Range (years)	Median (years)	Mean (years)
FFH	7 -75	41.0	41.8
PG	10 -88	31.5	35.3
POF	0 – 72	30.5	34.7
PGCG	13 -56	26.0	28.4

The frequency of the various FRGL and decade of life are shown in Figure 2. PG, POF and PGCG were more frequent in the 3rd decade of life, whilst the FFH was more common in the 4th decade (Figure 2).

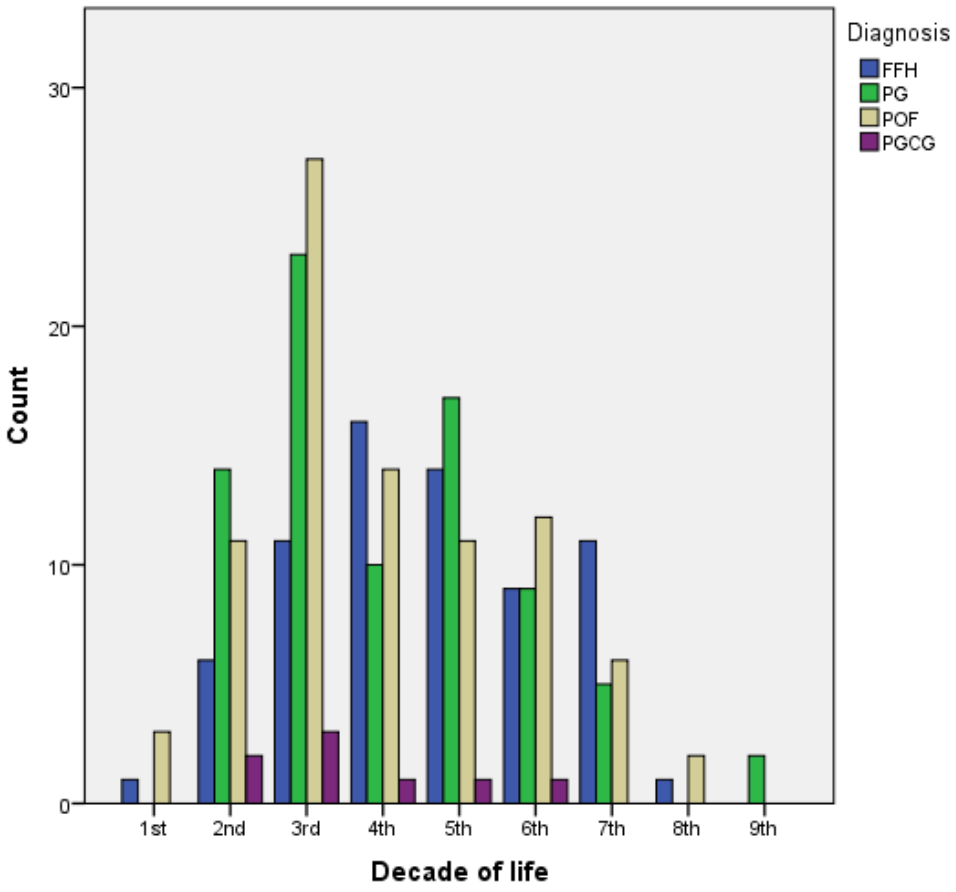

Figure 2: Frequency of FRGL and decade of life

The correlation between size of FRGL and age of patients was not statistically significant (Table 6).

Table 6: Correlation between size of FRGL and age

	Statistical test	**Age**	**P value**
Size of lesion	Rank biserial correlation	-0.28	0.661

3.4 Anatomic location

A total of 56.4% (n = 137) of the lesions occurred on the maxilla compared with 43.6% (n = 106) on the mandible. Of the 243 cases, 183 (75.3%) were located anteriorly (incisor-canine region) and 60 (24.7%) cases were located posteriorly (premolar-molar region). The anatomical distribution of the reactive focal lesions on the mandible and maxilla are shown in Figure 3.

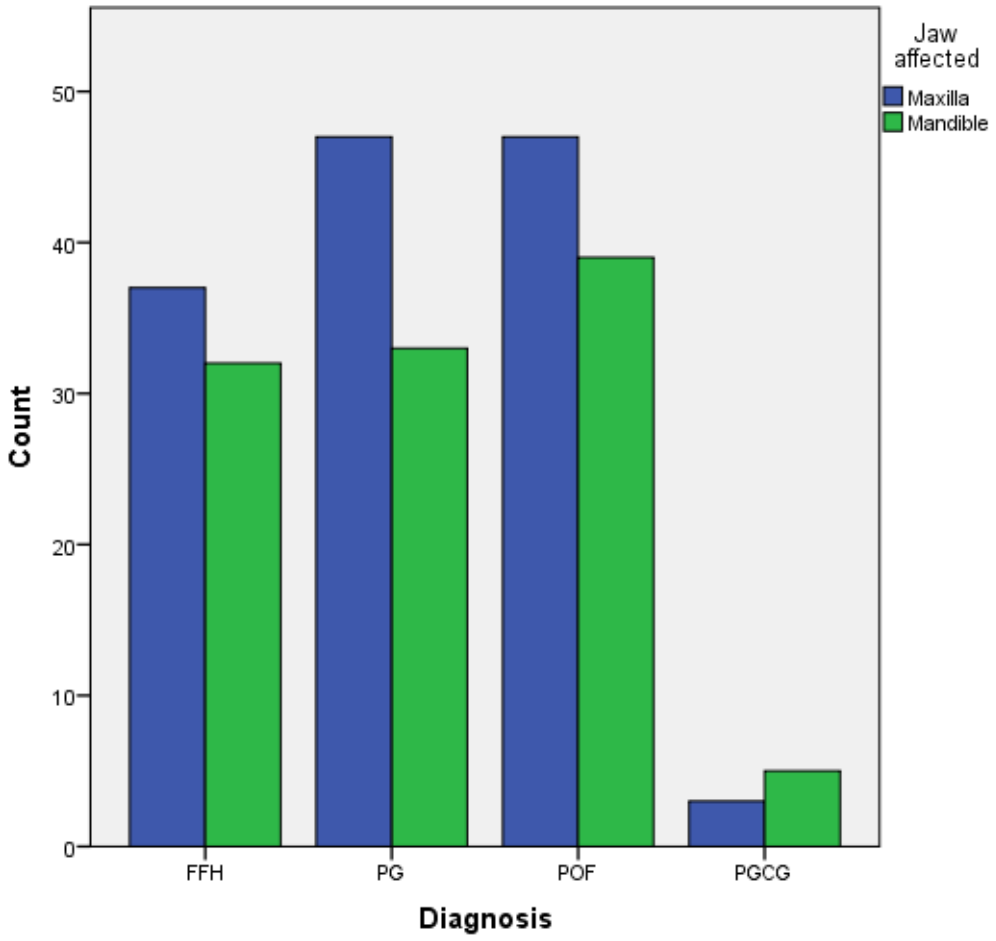

Figure 3: Distribution of FRGL on mandible and maxilla

The distribution of FRGL in the anterior (incisor-canine) and posterior (premolar-molar) jaws is shown in Table 7.

Table 7: Anatomical Distribution of FRGL

Diagnosis	Anterior maxilla	Posterior maxilla	Anterior mandible	Posterior mandible	Total	P value
FFH	30	8	21	10	69	0.978
PG	37	11	22	10	80	
POF	39	8	29	10	86	
PGCG	2	1	3	2	8	
Total	108	28	75	32	243	

The correlation of size of the FRGL and region of jaws affected (incisor-canine/ premolar-molar) yielded statistical significance (Table 8).

Table 8: Correlation of size of FRGL and region of jaws

	Region of jaws (anterior/ posterior)	P value
Diameter	0,126	0.05

3.5 Clinical features

A total of 11.1% (n = 27) of the lesions were associated with pregnancy (Table 9), whilst pedunculated lesions accounted for 51.9% (n = 126) of all lesions compared with 48.1% (n = 117) for sessile lesions (Table 10). A total of 60.1% (n = 146) of all lesions were erythematous (Table 11); and the correlation between the colour and the size of FRGL was statistically significant (P value = 0.0006) (Table 12).

Table 9: Distribution of FRGL in females according to pregnancy

Diagnosis	**Lesion during pregnancy**		**P value**
	Pregnant	**Not pregnant**	
FFH	4	44	
PG	15	42	
POF	8	54	0.044
PGCG	0	5	
Total	27	145	

Table 10: Distribution of FRGL according to the base of attachment

Diagnosis	Base of attachment		P value
	Pedunculated	Sessile	
FFH	29	40	0.005
PG	54	26	
POF	38	48	
PGCG	5	3	
Total	126	117	

Table 11: Distribution of FRGL according to colour

Diagnosis	Colour of lesion		P value
	Erythematous	Non-erythematous	
FFH	34	35	0.000002
PG	67	13	
POF	43	43	
PGCG	2	6	
Total	146	97	

Table 12: Correlation of size of FRGL and colour

	Statistical test	Colour of lesion	P value
Diameter	Rank biserial correlation	0.175	0.006

3.6 Recurrence of lesions

Recurrence rates of FRGL were 22.2% (n = 54) (Table 14); with no statistically significant difference in recurrence rates between males and females (Figure 4).

Table 13: Recurrence of FRGL

Diagnosis	Recurrence of lesion		Percentage recurrence (%)
	Recurrence	No recurrence	
FFH	12	57	17.4
PG	16	64	20.0
POF	24	62	27.9
PGCG	2	6	25.0
Total	54	189	22.2

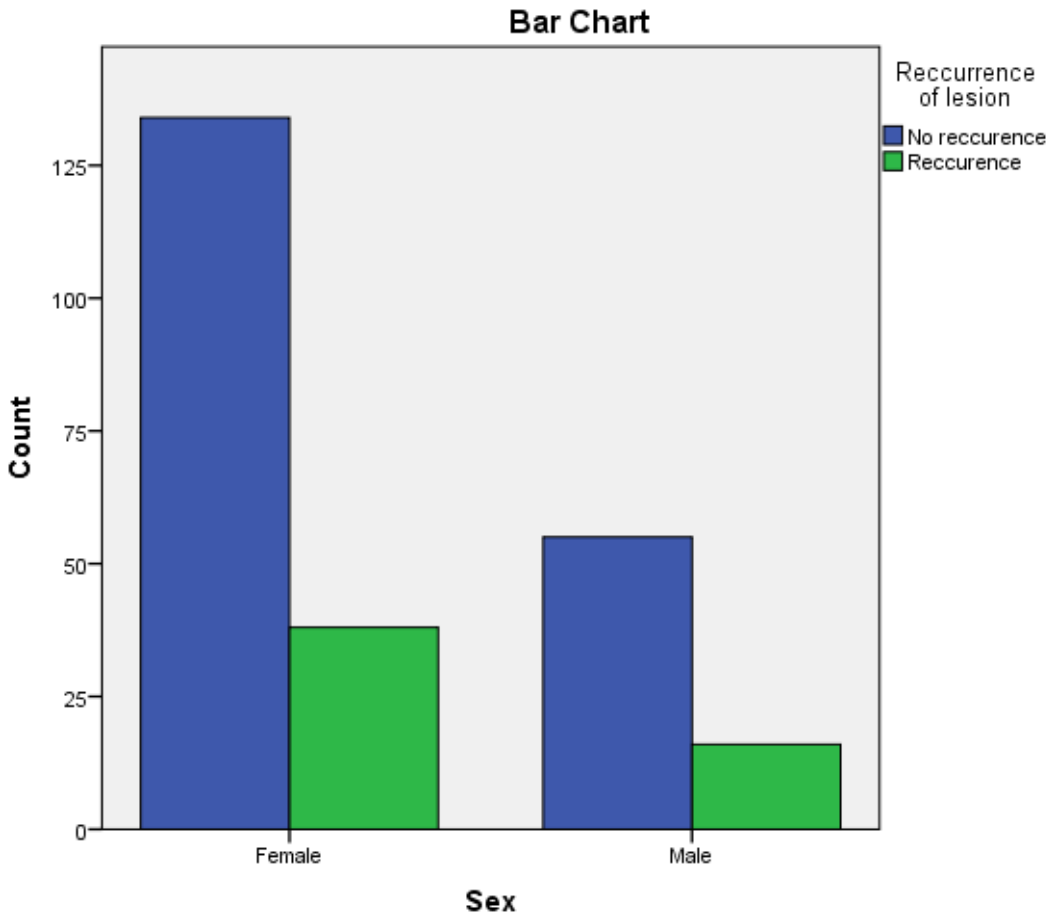

Figure 4: Recurrence rates according to sex

Chapter 4: Discussion

4.1 The frequency of FRGL

There is no consensus in the literature on which gingival variant is the most frequent FRGL; with most authors citing either the PG or FFH (Buchner et al., 2010, Daley et al., 1991, Effiom et al., 2011, Kfir, et al., 1980). In this study of 243 cases of FRGL, POF (35.4%, n = 86) was the most common, a feature not seen in any recent study utilising similar classification and methodology. The only comparative result found was by Macleod and Soames(1987) in England where POF constituted 40.5% of all gingival epulides. The variation noted in this study may be partly accounted for by differences in geographic and ethnic factors. Though some authors argue that there is bound to be a histopathological diagnosis overlap since FRGL represent a spectrum of one lesion, thus representing another possible source of the variation.

Studies in Chile and Western India, contrary to our findings, found the POF to be the least common FRGL with a frequency of only 2.9% and 9.7% respectively. (Maturana-Ramirez et al., 2015, Patil et al., 2014). In most countries the frequency of POF, ranged from 10% to 25%, representing the 2nd or 3rd most common focal reactive gingival lesion (Buchner et al., 2010, Effiom et al., 2011, Kfir et al., 1980, Zhang et al., 2007).

With a frequency of 32.9% the PG was the second most common reactive gingival lesion in this study. This agrees with most global studies that peg the frequency of PG between 25% and 35% of all FRGL (Buchner et al., 2010, Kfir et al., 1980, Maturana-Ramirez et al., 2015, Patil et al., 2014). However contrary to our results, a study in Nigeria found the PG to be the most frequent FRGL with a frequency of 57.0%.

The frequency of FFH across the globe is widely dispersed, ranging from 18.9% in Iran to 71.1% in Chile (Maturana-Ramirez et al., 2015, Zarei et al., 2007). The frequency of FFH in this study was 28.4% and is comparable to the 31.5% or 31.8% found in Jordan and Israel respectively (Ababneh, 2006, Buchner et al., 2010). PGCG was the least common FRGL in this study, a feature also seen in other studies (Dutra et al., 2018, Effiom et al., 2011, Maturana-Ramirez et al., 2015).

4.2 Distribution of FRGL according to sex

Numerous scholars have reported that FRGL have a predilection for females (Ababneh and Al-Dwairi, 2005, Bodner et al., 1997, Patil et al., 2014). This study found similar findings, with all four variants of FRGL occurring more commonly in females. Females accounted for 70.8% of all lesions which is comparable to 74.2% reported in Brazil, and marginally more than the 62.7% reported in Nigeria (Dutra et al., 2018, Effiom et al., 2011). The female hormones oestrogen and progesterone have been singled out as playing a key role in the aetiopathogenesis of FRGL (Kumar et al., 2006).

FRGL were significantly associated with pregnancy ($p = 0.044$), with most cases linked to the PG (55.6% of all pregnancy cases diagnosed as PG). This phenomenon was likely due to the vascular effects of female hormones on the gingiva (Dutra et al., 2018). No cases of pregnancy were associated with PGCG in this study, but this might be attributed to the lower numbers of FRGL analysed. A study that included 26 PGCG reported oestrogen sensitivity and hypothesised that growth of these lesions is partly governed by female sex hormones (Gunham et al., 1998).

4.3 Distribution of FRGL according to anatomical site

There was no statistically significant difference between frequency of FRGL in the maxilla and mandible. The frequency of FRGL in the maxilla was 56.4%, slightly higher than the results seen in Israel (52.2%) and Nigeria (50.0%) (Buchner et al., 2010, Effiom et al., 2011). Only the PGCG had a higher frequency in the mandible (62.5%), a trend which has also been seen in Nigeria, Israel, Sweden and Iran (Effiom et al., 2011, Buchner et al., 2010, Anneroth and Sirgurdson, 1983, Motamedi et al., 2007).

Most lesions were located anteriorly (incisor-canine region), a feature also seen in Brazil (Dutra et al., 2018). The pooling of saliva favours calculus formation which might result in gingivitis thus may partly explain this phenomenon.

4.4 FRGL and colour

There was a statistically significant relationship between FRGL and colour (p = 0.000002). PG was the lesion mostly associated with erythema, with 83.8% of all PG presenting as erythematous, slightly higher than the 74% recorded in Pakistan and 75% in Brazil (Kazmi et al., 2015, Peralles et al., 2006). The bulk of the PG lesion is angiomatous tissue formed from endothelial proliferation thus explaining the erythema (Neville et al., 2009). In this study 50.0% of POF were shown to be erythematous, a finding which is higher than the 36.0% and 33.4% recorded in Brazil and Pakistan respectively (Dutra et al., 2018, Kazmi et al., 2015). In this study 49.3% of FFH were erythematous which is comparable to the 55.1% reported in Pakistan (Kazmi et al., 2015). Only 25% of the PGCG in this study were erythematous compared to 61.8% reported in Pakistan.

The different amounts of hemosiderin or extravasated blood in various PGCG lesions results in a wide spectrum of colours at presentation that include red, bluish, pink and pale pink (Reddy et al., 2012). Since PGCG lesions were classified either as erythematous or non-erythematous rather than specify the true colour this might have been a source of error.

4.5 Distribution of FRGL according to size of lesions

In this study 74.9% (n = 182) of all FRGL were greater than 10mm, similar findings were also reported in Pakistan (Kazmi et al., 2015). The FRGL ranged from 4mm to 52mm, mirroring findings in Nigeria (Effiom, et al., 2011). Like other studies in Iraq and Nigeria, the average size of the PG was found to be the largest of all FRGL (Al-Rawi, 2008, Effiom et al., 2011). There was a low but statistically significant correlation between the size of FRGL and colour of the lesions (R_{rb} = 0.175), with erythematous lesions being generally larger. Low statistically significant relationships were also found between lesion size and location of lesion (anterior/posterior, R_{rb} = 0.126). Lesions tended to increase in size in an anterior-posterior direction, and this could be explained by the fact that patients are more likely to seek treatment earlier for anterior lesions because of a higher aesthetic demand in the incisor-canine regions. There was no correlation between size of lesions and age of patients (p = 0.661), with the largest lesion occurring in a 19-year-old female patient.

4.6 Distribution of FRGL according to age

The youngest patient in the study was 3 months old and was diagnosed with a POF. The baby was healthy and was born with anterior mandibular teeth that later exfoliated. Similar cases have been reported in literature, with the authors assuming the irritation in the periodontal ligament that results from exfoliation may trigger gingival connective tissue metaplasia with resultant dystrophic calcifications (Kohli et al., 1998, Yip and Sin Yeow, 1973). POF, PG and PGCG occurred more frequently in the 3rd decade of life, with similar results being reported in Nigeria and Israel (Buchner et al., 2010, Effiom et al., 2011). FFH was more common in the 4th decade, a feature also noted in Israel, though a study in Nigeria and Chile found lesions more common in the 3rd and 6th decade respectively (Buchner et al., 2010, Effiom et al., 2011, Maturana-Ramirez et al., 2015).

The mean age of patients with POF was 34.7 years, which is comparable to 33.9 years reported in Israel and 31.5 years reported in the USA (Buchner et al., 2010, Kfir et al., 1980). Though mean age for POF was noted to be way higher in China at 44.2 years (Zhang et al., 2007). The mean age for a PG was 35.3 years, with similar mean ages being reported in the USA and China (Kfir et al., 1980, Zhang et al., 2007). The lower mean ages for POF compared to PG does not corroborate the progressive development theory, that postulates that a POF evolves from of PG via further collagenisation and mineralisation (Prasad et al., 2008). Thus, this brings into question whether the PG and POF are the same lesion at various histopathological developmental stages.

FFH had the highest mean age at 41.8 years; similar results were also noted in the USA and China (Kfir et al., 1980, Zhang et al., 2007). The mean age of PGCG was 28.4 years, which was somewhat lower than the USA (39.6 years) and Israel (43.2 years) (Buchner et al., 2010, Kfir et al., 1980).

There was no statistically significant difference in recurrence rates for FRGL in this study. The recurrence rate of 22.2% recorded in this study is higher than the 2.9% reported in Nigeria (Effiom et al., 2011). The POF and FFH had the highest and lowest recurrence rates respectively, a feature also reported in India (Babu & Hallikeri, 2017). There was also no statistically significant difference in recurrence rates between males and females, hence female hormones may not be key to recurrence rates as removing chronic gingival irritation

after resection or cutting the reactive lesion with the associated periodontal ligaments (Babu and Hallikeri, 2017).

4.7 Limitations of study

The study fell victim to a major hurdle of retrospective studies, which is, all information gathered is solely dependent on the available information in the dental records. Unfortunately, this resulted in insufficient data being gathered on race, plaque index, DMFT index, nature of chronic irritation and presence of ulceration as per requirement of the study. Pregnancy status evaluation was solely based on information gathered on the dental chart, thus some early unknown pregnancies might have gone undetected.

Chapter 5: Conclusion

Non-neoplastic masses are extremely common in the oral cavity, particularly the gingiva (Effiom et al., 2011). As in most studies the FRGL were more common in females in this study. After analysing 243 cases it was noted that unlike most countries the POF was the most common FRGL. Ethnic and geographic differences may account for the variation noted. No statistically significant differences were noted with FRGL when the mean age of patients, jaw affected, anatomic location of lesion, size of lesion and sex. Statistical differences for FRGL was noted when looking at pregnancy, base of attachment (sessile and pedunculated) and colour of lesions. An understanding of the clinical picture may aid in diagnosis of FRGL, but histology is still required for a definitive diagnosis.

Retrospective studies heavily dependent on data available in patient records. Given the amount of missing data encountered in the study, we recommend detailed and accurate documentation of findings by clinicians in order to facilitate future research.

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Chapter 6: Annexures

Annexure A: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr C Karuma

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180672

NAME: (Principal Investigator)	Dr C Karuma
DEPARTMENT:	School of Oral Health Sciences Department of Oral Pathology Medical School University
PROJECT TITLE:	Clinicopathological evaluation of focal reactive lesions of the gingiva
DATE CONSIDERED:	29/06/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr S Ngwenya and Professor SL Shangase
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/10/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore reports and re-certification will be due early in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Annexure B: School of Oral Health Science permission letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

School of Oral Health Science

Department of Oral Biological Sciences, 7 York Road, Parktown, 2193. Tel: 011 717 2045 Fax: 086 553 3890 Email: Julitha.Molepo@wits.ac.za

19 September 2018

Dr C Karuma
29 Earls Avenue
Windsor West
Johannesburg

RE: PERMISSION TO CONDUCT A RESEARCH

Your request for permission to conduct a research has been provisionally approved by the Committee.

The Hospital Research and Risk Committee is requesting you to provide Patient Admin Manager Mr. Bushi Ramoleta with the list of patients files you want access to.

A list should contain the following info:
1. File number
2. Patient name and surname

Regards,

Prof MS Nemutandani
CEO/Head of School
Date: 19/09/2018

Annexure C: Department of Oral Medicine and Periodontology permission letter

Department of Oral Medicine and Periodontology

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

School of Oral Health Sciences
Queens Road
Parktown, Johannesburg

Area 3 -8th Floor - room 385-31
C.M. Academic Johannesburg Hospital

Private bag 3
2050, Wits, South Africa
+27 011-488 4889/7
+27 11 488 - 4902
E-mail: Sindiswa.Shangase@wits.ac.za
Matsie.Sibeko@wits.ac.za

July 18th, 2018

Dear Dr C Karuma

Re: Request to access Oral Medicine patient records

Permission to access records of Oral Medicine patients in the Department of Oral Medicine and Periodontology is hereby provisionally granted pending ethical clearance from HREC. This permission covers the applicant solely for purposes of data collection towards a research project titled, ': *Clinicopathological evaluation of focal reactive lesions of the gingiva'*, pending approval from the Research Ethics Committee.

Yours sincerely,

Prof. S.L. Shangase
Head of Department

Annexure D: Department of Oral Pathology permission letter

Department of Oral Pathology
School of Oral Health Sciences
Faculty of Health Sciences
3E22, 3rd floor, Wits Medical School
7 York Road, PARKTOWN, 2193
Private Bag 3, Wits 2050, South Africa
Tel: 011717213997
Fax: 0117172146
Email: HOD: Sizakele.Ngwenya@wits.ac.za
Secretary: Phindile.Mashinini@wits.ac.za

01 June 2018

Professor P Cleaton-Jones
Chairperson of the Human Research Ethics Committee (Medical)
Research Office
Faculty of Health Sciences
University of the Witwatersrand

Dear Prof Cleaton- Jones

RE: PERMISSION TO CONDUCT A STUDY IN THE DEPARTMENT OF ORAL PATHOLOGY

I, **Dr Sizakele P Ngwenya**, in my capacity **as Head of the Department of Oral Pathology grant Dr Cliff Karuma permission** to access the Department's histopathological reports to retrieve demographic and clinicopathological data as specified in his data collection sheet for his study entitled:

Clinicopathological evaluation of focal reactive lesions of the gingiva.

Yours Sincerely,

DR SP NGWENYA
Head: Department of Oral Pathology
School of Oral Health Sciences
Faculty of Health Sciences
University of the Witwatersrand
Private Bag 3, WITS, 2050

Annexure E: Budget

Printing expense	Number of pages	Number of copies	Cost (ZAR)
Ethics application	10	25	50
Protocol	16	5	80
Research report	60	5	300
Total	76	15	430

Research was self-funded.

Annexure F: Data collection sheet

Case Number : **Year:** **Histopathological diagnosis:**

Variable		**Finding**
Age		
Sex		
Race		
Occurrence of lesion during pregnancy		
History of:	**Chronic irritation**	
	If yes, specify source:	
	Calculus	
	Ill-fitting dental appliance	
	DMF index	
	Poor oral hygiene	
	Plaque index	
Recurrence of lesion		
Diameter of lesion		
Pedunculated/ sessile		
Colour of lesion		
Jaw affected		
Region of jaw affected		
Presence of ulceration		
Bone loss		