Clinicopathological and Immunohistochemical Analysis of 21 cases of Traumatic Ulcerative Granuloma with Stromal Eosinophilia Using CD30, CD68 and TGF-β1

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ABSTRACT

Background: Traumatic ulcerative granuloma with stromal eosinophilia is an impressive benign chronic ulcerative lesion of the oral mucosa with vague etiopathogenesis. It was supposed to represent an oral counterpart of primary cutaneous CD30+ lymphoproliferative disorder. Histopathologically, it is characterized by mixed inflammatory infiltrate predominated by histiocytes, lymphocytes and eosinophils along with presence of scattered large atypical mononuclear cells. It has worrisome clinical presentation. It may heal spontaneously, but in most occasions it persists and never heal unless removed surgically (incisional or excisional biopsy). A rare subset may show worrisome immunohistochemical features. Follow up is highly recommended.

Materials and methods: Formalin fixed - paraffin embedded tissue blocks of twenty-one cases were cut and mounted on positively charged slides and stained by primary antibodies (CD30, CD68 and TGF-β1). A statistical analysis was performed between the immunohistochemical scores for markers with each other and with clinicopathological parameters (age, sex, size of ulcer, number of eosinophils and mitoses).

Results: The age of the patients ranged from 20 to 72 years, with a higher female propensity. Immunohistochemical positive expression for CD30 (16 case) mainly involved round small lymphocytes, while all cases were positive for CD68 and TGF-β1. Statistically, there was no significant relation between the scores of CD30, CD68 and TGF-β1 with each other and with the aforementioned parameters, ($P<0.05$). The eosinophils count showed a significant positive correlation with age ($P=0.008$), size of ulcer ($P=0.007$) and mitoses ($P=0.004$).

Conclusion: Traumatic ulcerative granuloma with stromal eosinophilia is a benign and reactive chronic oral ulcerative lesion rather than being CD30+ lymphoproliferative disorder; this conclusion is supported by heterogeneous, focal and nonspecific staining for CD30 and being typically infiltrated by CD68+ macrophages. Whereas, a high level of expression for TGF-β1 indicated that the aforementioned factor was not associated with the delayed healing of this lesion. **(Received: 12/9/2018; Accepted: 17/10/2018)**

INTRODUCTION

Traumatic ulcerative granuloma with stromal eosinophilia (TUGSE) is an oral ulcerative lesion of much interest for many medical specialties (oral pathology, dermatology, dentistry, surgery, and otolaryngology); so that, various terms have been used to identify this lesion[1]. The story of appellation began in the fifties of the 20th century until the 1st decade of 21st century. At the first attempts, TUGSE was erroneously called xanthogranuloma, nevoxanthoendothelioma, juvenile xanthoma and eosinophilic granuloma[2]. Thereafter, it was termed traumatic granuloma of the tongue[2]; ulcerated granuloma eosinophilicum diutinum of the tongue[3]; eosinophilic granuloma of the tongue[4]; eosinophilic ulcer of the tongue[5]; traumatic eosinophilic granuloma of the gingiva[6]; ulcerative eosinophilic granuloma of the tongue[7]; traumatic ulcerative granuloma with stromal eosinophilia (TUGSE)[8]; eosinophilic ulcer of the oral mucosa[9] and abbreviated as (EUOM)[10]; ulcerative eosinophilic granuloma (UEG)[11]; traumatic eosinophilic granuloma (TEG) of the oral mucosa[12]; and oral traumatic granuloma (TG)[13].

Unknown", "Poorly understood", "Not clear", "Debatable", "Obscure", "Unclear", "Uncertain", "Controversial", all of these terms have been used to illustrate the etiopathogenesis of TUGSE. Until nowadays, the etiopathogenesis of TUGSE is a matter of controversy and the enigma of TUGSE etiopathogenesis is yet be unraveled. Among all etiological factors, mucosal trauma appeared to be the major instigating factor of this lesion[2, 14]. However, it was assumed that if the trauma was the sole cause, TUGSE would be more common; therefore, it was suggested that viral or toxic agents might enter into the underlying tissue and result in an inflammatory response and tissue damage[7]. Then, virus-related etiopathogenesis was suggested[15, 16]. However, the possibility of viral-mediated etiopathogenesis was discarded[17-20]. Furthermore, a hypothesis of cell-mediated pathogenesis was suggested[10, 21]. With a diverse point of view, it was proposed that the chronicity of TUGSE might be caused by an underlying defect in the healing process that resulted from a

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lack of expression of transforming growth factor-α (TGF-α) and transforming growth factor-β (TGF- β) by eosinophils infiltrating such a lesion[22].

Clinically, TUGSE is characterized as a solitary benign chronic oral ulcer, with the tongue being the most common site to be affected; however, it may present elsewhere in the oral cavity such as lips, gingiva, palate, vestibular mucosa, retromolar area and floor of the mouth[14, 21]. It has an important clinical significance, since it may be provisionally diagnosed as oral squamous cell carcinoma (OSCC) because of its worrisome clinical presentation as a chronic oral ulcer with elevated and rolled margins that fails to heal by means of local treatment[23].

Histopathologically, TUGSE is presented as an ulcerated lesion composed of a poorly formed granulation tissue showing a mixed inflammatory infiltrate that is composed of histiocytes, lymphocytes, eosinophils, plasma cells and large atypical mononuclear cells; the eosinophilic infiltrate in TUGSE lesions is characteristic and fundamental for the diagnosis, since most of the oral traumatic ulcers are devoid of such heavy eosinophilic infiltrate[23]. The inflammatory infiltrate affects the superficial and deep layers of the muscular tissue and penetrates into underlying soft tissue. Large atypical mononuclear cells with ovoid and pale-appearing nuclei are scattered and may be mitotically active[24].

By immunohistochemistry, it was revealed that the cells that made up the characteristic infiltrate of large round cells expressed the macrophage marker (CD68) or the dendrocyte marker (factor XIIIa)[25], the lymphocytic infiltrate was composed predominantly of T cells[10]. Interestingly, TUGSE was suggested to represent an oral counterpart of cutaneous CD30+ lymphoproliferative disorder (LPD)[12, 18]. While on the basis of molecular and immunohistochemical features, it was quite conceivable to suggest that TUGSE symbolized an umbrella term covering a spectrum of lesions with diverse cells of origin[17].

This study aimed at the assessment of clinicopathological and immunohistochemical features of TUGSE to reach a favorable consensus about the nature, behavior, etiopathogenesis, cellular characteristics and optimal diagnostic criteria of such mysterious oral ulcerative lesion. The immunohistochemical markers in this study were (CD30, CD68 and TGF-β1).

MATERIALS AND METHODS

Formalin fixed-paraffin embedded tissue blocks (incisional and excisional biopsies) of twenty-one cases of TUGSE were retrospectively retrieved. The diagnosis was made according to the criteria in Table (1). Tissue sections (5µm) were cut and mounted on positively charged slides and stained immunohistochemically with polyclonal antibodies to CD30 (ab203593, 1:100), CD68 (ab203101, 1:200) and TGF-β1 (ab92486, 1:200) using EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436, 15ml).

Immunohistochemical signal specificity was demonstrated by the presence of a brown granular DAB staining pattern within the specific tissue compartment for a certain antibody in positive control tissue sections according to manufacturer's datasheets, and the absence of such staining in negative controls tissue slides.

Five representative fields were selected for each tissue section in all primary antibodies, visualized and scored microscopically with a 400X objective; the average percent of the five high power fields was calculated for each marker. All cases were blindly evaluated without prior knowledge of the other parameters.

The immunohistochemical staining for CD30, CD68 and TGF-β1 antibodies was measured semi-quantitatively and assigned into categories for each one, as follows:

CD30 scoring: 0 (none); 1 (less than 30%); 2 (30% to 50%); 3 (more than 50%)[24].

CD68 scoring: 0 (none); 1 (less than 25%); 2 (25% to 50%); 3 (50% to 75%); 4 (more than 75%)[21].

TGF-β1 scoring: 0 (0% to 10%); 1 (10% to 25%); 2 (25% to 50%); 3 (more than 50%)[26].

Table 1: Diagnostic criteria that were considered for diagnosis of TUGSE in the current study.*

Diagnostic criteria of TUGSE
Clinical criteria:
✓ Almost persistent ulcer, sometimes with raised and indurated borders.
✓ Resistance to local treatment for at least 2 weeks.
✓ Implication of antecedent trauma is usual, but not mandatory.
Histologic criteria:
✓ Ulcerated mucosa with surrounding epithelium that is usually hyperplastic.
✓ Underlying connective tissue is infiltrated by mixed inflammatory cells mainly histiocytes, lymphocytes admixed with increased number of eosinophils (poorly formed granulation tissue).
✓ Stromal eosinophilia is a must diagnostic criterion.
✓ Presence of large atypical cells with pale staining nuclei admixed with the inflammatory infiltrate.
✓ Extension of such infiltrate to deep structures (skeletal muscle fibers and salivary glands).
✓ Skeletal muscle fibers show signs of regeneration and degeneration.
✓ Presence of mitoses is not uncommon
Immunohistochemical criteria:
✓ Positive expression of CD68 marker is must.
✓ Heterogeneous positive expression of CD30 marker, which may be absent in some cases.

* The diagnostic criteria were derived from the data of previous literature and observations in the current study.

RESULTS

Clinical description:

The age of the patients ranged from 20 to 72 years old with a mean of 53 years and a higher incidence at the fifth to seventh decade. Data regarding sex distribution among cases revealed a higher female propensity (15/21, 71.4%) than that for males (6/21, 28.6%), with male-to-female ratio was equal to 1:2.5. Regarding site affected, the tongue (especially the dorsum and lateral borders) was involved in all but one case, which involved the upper lip. The data regarding the size of the ulcers were gathered from clinical information of some cases and measured from the received surgical specimen of the rest of cases; however, it ranged from 0.2cm to 1.56cm with a mean of 1.03cm. Information about the duration of the lesion was lacking in a half of cases; however, available data revealed that the duration ranged from 1 month up to 1 year with a mean of 3.2 months.

Gross and histopathological findings:

The gross examination of the specimens of TUGSE (incisional and excisional biopsies) revealed whitish, grayish, whitish-gray or grayish-white soft tissue lesions. The size of specimens ranged from 0.4cm to 4cm in diameter, where some excisional biopsies included the ulcerated area with a safe margin because of suspicion of OSCC (Fig. 1).

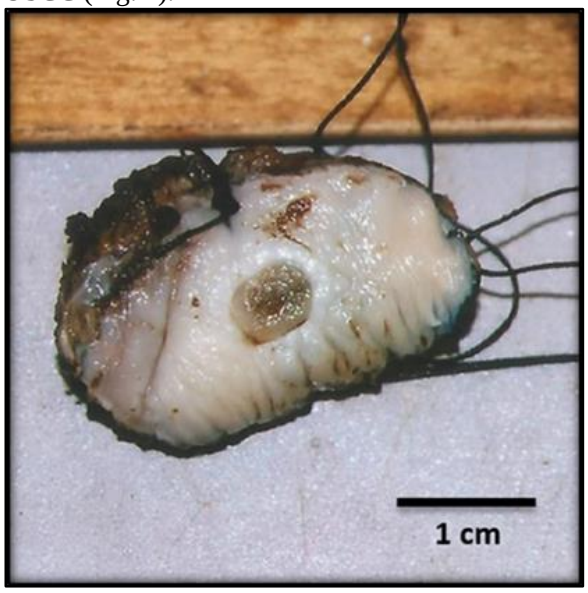

Figure 1: Cut section of an excisional biopsy of TUGSE at the lateral border of the tongue, one suture refer to superior border and double sutures refer to anterior border (specimen included safe margin resection).

Microscopically, sections showed ulcerated stratified squamous epithelium. The underlying stroma showed a mixed chronic inflammatory cell infiltrate composed mainly of histiocytes, lymphocytes, and varying numbers of eosinophils in between degenerative-regenerative skeletal muscle fibers (Fig. 2). However, other cellular components such as mast cells and plasma cells were also present, but in smaller proportions, in addition to few scattered large atypical cells.

The presence of eosinophilia is characteristic of TUGSE, where the other oral traumatic ulcers are devoid of such a tissue eosinophilia. The number of eosinophils for each case was counted and ranged from 10 to 50 eosinophilic leukocyte per 10 high power fields (HPF). Mitoses were present in 11 cases with the highest count was equal to two mitotic figures per 10 HPF (Fig. 3).

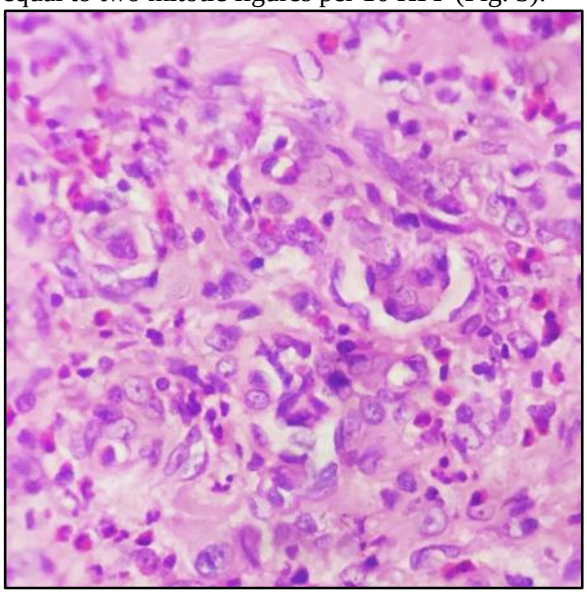

Figure 2: High power photomicrograph of TUGSE showing a mixed chronic inflammatory cell infiltrate composed mainly of histiocytes, lymphocytes, and eosinophils (H&E, X400).

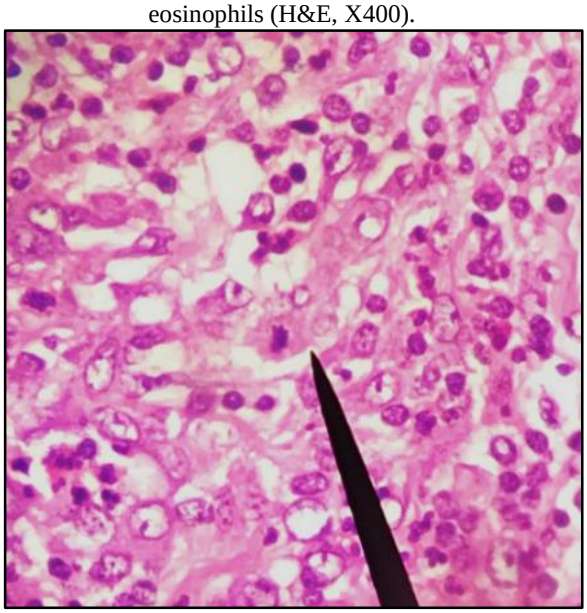

Figure 3: Photomicrograph of TUGSE showing a mitotic figure (at the tip of the pointer) (H&E, X400).

Regarding tissue eosinophilia, there was a significant positive correlation between

eosinophils count and age (P=0.008) and size (P=0.007); while, there was no significant correlation with duration (P=0.495). Additionally, there was no significant difference in eosinophils count between males and females (P=0.709). While for mitoses, there was neither significant correlation between mitosis and age (P=0.263), size (P=0.122) and duration (P=0.805) nor significant difference in mitosis between males and females (P=0.522).

Immunohistochemical Findings:

The expression of CD30 was positive in 16 case (>75% positive cells in three cases) and mainly involved the round small lymphocytes, but some of the large atypical cells were, also, CD30 positive (Fig. 3, a). Statistically, CD30 had no significant relation with sex (P = 0.216). Similarly, there was no significant difference in CD30 score groups with age (P=0.357), duration (P=0.717) and size (P=0.171).

All cases were CD68 positive with positivity varied from 9.7% up to 97.8% as brown membranous staining of histiocytic mononuclear cells (Fig. 3, b). Statistically, there was no significant relation between CD68 scores and sex (P=0.251); also, there was no significant difference in CD68 score groups with age (P=0.566), duration (P=0.205) and size (P=0.758).

Cytoplasmic, nuclear and/or extracellular matrix TGF-β1 positive expression were demonstrated in stromal tissue sections of all cases (Fig. 3, c). In relation to demographic parameters, there was no significant relation between TGF-β1 and sex (P=0.347). Furthermore, there was no significant difference in TGF-β1 score groups with age (P=0.072), and size (P=0.689), but there was a significant difference in the duration (P<0.05).

Correlations among Immunohisto-chemical Markers:

Using Pearson's correlation, no significant correlations were evident between immunohistochemical markers studied; TGF-β1 and CD30 score groups (P=0.347); TGF-β1 and CD68 score groups (P=0.390); CD30 and CD68 score groups (P=0.117). However, there was a positive significant correlation between and mitoses (P=0.004), while there was no significant correlation of eosinophils count with the other immunohistochemical markers (P>0.05).

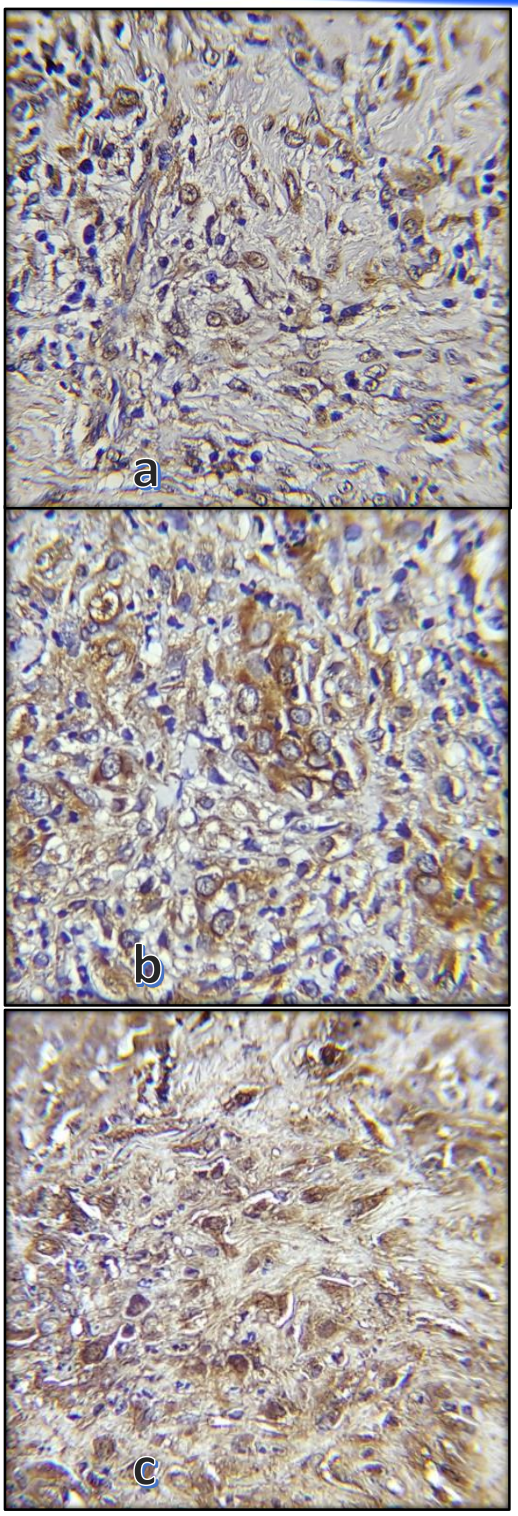

Figure 4: High power photomicrograph showing the positive immunohistochemical expression of primary antibodies: a. CD30; b. CD68; c. TGF-β1.

DISCUSSION

Generally speaking, TUGSE is not an uncommon oral lesion, but in Iraq, it seems to be considered as a rare and recently delineated oral lesion that might be reflected by the general lack of awareness of this entity. However, the final sample of this study was comprised of 21 cases

that seems to be adequate to represent a reliable result. To the best of our knowledge, this study is the 5th largest series of cases of TUGSE in English language literature.

All age cohorts can be affected; it has two peaks of incidence: the first peak occurs in children, mostly being related to eruption of primary anterior teeth that is referred to as Riga-Fede disease (RFD)[8], while the second peak occurs in adults. The age of the sample in this study was in accordance with majority of the main series of cases of TUGSE (Table 2).

On the basis of data available in the literature, no dominant sexual predilection was apparent; male predominance[2, 30], female predominance[10, 20, 25, 28, 32] and equal male-to-female ratio[8, 21, 27, 29] have been reported (Table 2). However, fluctuations of male-to-female among different series of cases of TUGSE necessitate further studying of this condition to reach a reasonable sequel.

Variation in duration might be attributed to that information was limited to estimations given by patients that might not be precise and affected by socioeconomic status of patients, where TUGSE may be presented as a painless lesion that can be neglected by some patients for a long period of time; however, it was consistent with many other studies of TUGSE (Table 2).

Regarding the site affected; in this study, the tongue (especially the dorsum and lateral borders) was involved in all but one case, which involved the upper lip. Similarly, the tongue was stated as the commonest site in all previous studies of TUGSE, with lip localization has been reported in few instances (Table 2).

Trauma is considered as an inevitable cause related to TUGSE etiopathogenesis. Unfortunately, this study lacked information about presence of history of trauma in 14 cases, with only 4 cases were reported with obvious previous source of traumatism (irritation from sharp root stumps or a badly carious tooth). Accordingly, such high affinity of tongue involvement, as the most common site of TUGSE, seems reasonable since the tongue movement makes it more vulnerable to trauma. However, trauma, per se, could not be considered as a sole cause of TUGSE, where in this study, 3 cases reported without known history of trauma. Similarly, both states of being traumatized or not have been reported by other authors (Table 2).

In the context of the size of ulcer, the results were nearly identical to those obtained by Hirshberg et al.[21] and Jayalakshmy et al.[32], slightly greater than those addressed by Abdullah[29], while, in other studies[10, 27], the ulcers had much greater diameter; per contra, the rest if studies of TUGSE lacked information about such parameter (Table 2).

By examining hematoxylin and eosin (H&E) tissue sections, all cases in this study showed an ulcerated oral mucosa with mostly hyperplastic edges. The underlying stroma showed a mixed chronic inflammatory infiltrate comprised mainly of histiocytes, lymphocytes, mast cells, plasma cells and varying numbers of eosinophils in between degenerative-regenerative skeletal muscle fibers. Additionally, large atypical cells have been found in varying numbers and distribution. Mitoses were present in about half of cases. Almost all previous series of cases of TUGSE (Table 2) showed similar histopathological features.

Being correspondent with other studies of TUGSE, the eosinophilic infiltrate in this study was of varying densities among different cases and within the same case in different areas of the section; however, the eosinophils count in the current study was in agreement with findings of other authors, where a significant degree of tissue eosinophilia was recorded when it was possible to find more than ten eosinophils per HPF[27, 30].

The presence of tissue eosinophilia is not completely understood because most of oral traumatic ulcers are devoid of such increased eosinophils; however, such stromal eosinophilia may represent a tissue reaction to unknown antigens introduced through mucosal breakdown following trauma[21]. Furthermore, mucosal degeneration that is so characteristic of TUGSE may be attributed to toxic products released by degranulating eosinophils[21]. However, the tissue eosinophilia might be resulted from release of cytokines from T-lymphocytes[10], or due to release of eosinophilic chemotactic factors by mast cells[8].

Regarding statistical analysis of tissue eosinophilia, there was a significant positive correlation between eosinophils count with age (P=0.008) (supporting the higher incidence of TUGSE in advancing age, namely fifth to seventh decades of life) and with size (P=0.007) (as eosinophils react to macroorganisms, foreign antigens, viruses and other tissue breakdown products that will be, conveniently, increased with increasing size of its portal of entry, the ulcer). So that, besides that stromal eosinophilia is a characteristic feature of TUGSE, it is suggested here that the presence of tissue eosinophilia in sections of oral lesions is almost never nonspecific.

The presence of mitoses is another interesting feature of the cellular infiltrate of TUGSE, but not an inherent finding that has been reported variably. In this context, 10 cases in this study

were mitosis-free, while, in the rest of cases, mitoses were identified in scattered cells and were not abundant with highest count was equal to 2 mitotic figures per 10 HPF. In comparison with other studies in literature, many of them were lacking to evaluate this parameter; nevertheless, the instances, in which mitosis was mentioned, were in accordance with the results of this study[2, 10, 28, 34]. According to the positive significant correlation between eosinophils count and mitoses (P=0.004), it is suggested that the higher eosinophils count, the higher the proliferative capability of TUGSE that necessitate awareness and regular follow up.

Previously, TUGSE was suggested to represent an oral counterpart of cutaneous CD30+ LPD[12]. In this context, Alobeid et al.[17] reported 3 cases of TUGSE lesions that were strongly positive for CD30 and showed a monoclonality, suggesting TUSGE to represent a heterogeneous category of disorders including CD30+ LPD[17]. Furthermore, a proposal of mucosal CD30+ LPD was aroused again through a study of 4 cases of oral ulcerative lesions that supposed to represent TUGSE, where all cases showed CD30 positivity with the presence of T-cell monoclonality[18].

On the other hand, TUGSE was considered as a reactive oral lesion in a study of 12 cases, but might harbor a dominant clonal T-cell population; CD30 expression was evident only in 5 cases[21]. Similarly, a reported case of recurrent CD30+ TUGSE, a reactive nature was suggested[35]. Later on, a reactive nature of TUGSE was also postulated[36, 37]. In rejecting the concept that TUGSE represented the oral counterpart of primary cutaneous CD30+ LPD, a study of 37 cases of TUGSE showed no specific relation between the presence of CD30+ large atypical mononuclear cells and the presence of T-cell monoclonality[28]. In their study, Fonseca et al. stated that those lesions behaved in a benign and reactive way [24].

In the current study, the expression of CD30 was heterogeneous with focal and nonspecific staining pattern. Positivity was exhibited mainly by small round lymphocytes with presence of some CD30+ large atypical cells indicating that almost all lesions of TUGSE in this study were benign and reactive; but, it is worth to mention that in spite of designating TUSGE as a benign and reactive ulcerative lesion of oral mucosa, a rare subset of TUGSE may show worrisome immunohistochemical features (presence of high level of CD30 expression as in 3 cases in this study, >75%) and molecular findings (evidence of monoclonality)[17, 18, 21, 28]; regarding the latter, it is suggested that the continuous irritant insult could eventually affect cellular differentiation from polyclonal toward oligoclonal then monoclonal that is proposed to be similar, in terms of pathogenesis, to gastric mucosa-associated lymphoid tissue lymphoma (MALToma) and immunoproliferative small intestinal disease (IPSID)[38].

Regarding the expression of CD30 marker, the results of this study were in accordance with many other studies in postulating TUGSE to be a benign and reactive ulcerative lesion of the oral mucosa[35-37] that may show some worrisome features such as high level of CD30 positivity and T-cell monoclonality[21, 24, 28], but disagree with those who supposed TUGSE to represent an oral counterpart of cutaneous CD30+ LPD or primary mucosal CD30+ LPD[12, 17, 18].

All cases showed CD68 positive histiocytes with varying expression from one case to another. These results were in accordance with the vast majority of previous series and case reports of TUGSE (Table 3), but disagreed with Alobeid et al.[17] who stated negative CD68 expression in their 3 cases reported. It is suggested that TUGSE is typically infiltrated by histiocytic macrophages (CD68+) which represent one of the most dominant cellular infiltrate of chronic inflammatory responses; so that, CD68 marker has to be considered as a diagnostic criterion for TUGSE. Rather than being a simple wound that, for one reason or another, fails to heal, TGUSE is supposed to represent a specific oral chronic lesion with peculiar features, since a subset of TUGSE may be presented with unusual and alarming signs.

Regarding TGF-β1 expression, the only study that assessed the expression of the aforementioned TGF was carried out by Elovic et al.[22] who stated that eosinophils infiltrating TUGSE lesions expressed little or no TGF-α and TGF-β1 and the delayed healing of TUGSE was attributed to such lack of expression; however, other cells such as epithelial, mononuclear and fibroblasts did express these cytokines in their study[22]. The present study is the second study in evaluation of TGF-β1 in TUGSE lesions where all cases were highly positive. Anyhow, in contradiction with the hypothesis of Elovic et al.[22], it is suggested that delayed healing of TUGSE may be imputed to reasons other than the lack of TGF-β1 expression by eosinophils. Therefore, it is not fair to neglect TGF-β1 expression by other cellular components in TUGSE.

Table 2: Large series of cases of TUGSE.

Author	Number of cases	Mean age (range)	Sex (M/F)	Size (mean)	Duration	Previous trauma
Bhaskar and Lilly [2]	7	37 (20-59)	2.5/1	Not stated	14–63 days	None
Elzay [8]	41	58 (14-92)	1/1	Not stated	3–120 days	21 cases
Doyle et al. [27]	15	62 (42-77)	1.1/1	0.3- 5 cm (1.8)	2 weeks-6 months	5 cases
El-Mofty et al. [10]	38	57 (6-88)	1/1.5	0.5- 6.5 cm (2.2)	Weeks to months	7 cases
Regezi et al. [25]	8	59 (10-87)	1/3	Not stated	2 weeks-6 months	Not stated
Elovic et al. [22]	12	62.2 (38-85)	1/1.6	Not stated	2 weeks- 8 months	1 case
Hirshberg et al. [21]	12	49.2 (14-87)	1/1	0.3-1.5cm (0.9)	Days to 1 year	4 cases
Salisbury et al. [28]	37	58.1 (11-91)	1/2	Not stated	Days to years	Not stated
Abdullah [29]	17	40 (16-70)	1/1.1	0.25- 2 cm (0.5)	1 month- 2 years	Not stated
Fonseca et al. [24]	19	58.6 (35-84)	1.3/1	Not stated	2 - 48 months	7 cases
Shen et al. [30]	34	49 (8-80)	1.8/1	Not stated	Not stated	Not stated
Kaplan et al. [31]	16	60	1.5/1	Not stated	Not stated	4 cases
Jayalakshmy et al. [32]	6	60.3(53- 77)	1/5	0.6- 1.5 cm (1.08)	Not stated	4 cases
Phoorisriphong et al. [33]	8	59.1 (10-86)	3/1	Not stated	Not stated	Not stated
Vargo et al. [20]	6	60.5 (53-74)	1/5	Not stated	Not stated	2 cases
Current study	21	53 (20-72)	1/2.5	0.2-1.56 cm (1.03)	1 month- 1 year	4 cases

Table 3: Studies and case reports examining the expression of CD68 in TUGSE.

Author	Number of cases	CD68 expression	Comments
Regezi et al. [25]	8	+ve	The large round cells expressed the macrophage marker, CD68.
El-Mofty et al. [10]	9 of 38	+ve	CD68+ histiocytic cells were less common than T-cell markers.
Ficarra et al. [12]	1	-ve	The infiltrate composed of T-cells with CD1a+ dendritic cells.
Horie et al. [34]	1	+ve	Focal expression for CD68.
Alobeid et al. [17]	3	-ve	The neoplastic cells were negative for CD68 protein.
Hirshberg et al. [21]	12	10 +ve	CD68+ cells were found in most cases.
Segura and Pujol. [36]	1	+ve	Abundant CD68+ histiocytes throughout the lesion.
Boffano et al. [37]	1	+ve	Diffuse positivity of the histiocytes for CD68 was demonstrated.
Vasconcelos et al. [39]	1	+ve	Diffuse immunoreactivity for CD68 in the inflammatory cells, defining these cells as macrophages and not as neoplastic cells.
Bortoluzzi et al. [40]	1	+ve	Diffuse positive pattern of CD68.
Brasileiro et al. [19]	1	+ve	CD68 evidenced numerous reactive histiocytes.
Chatzistamou et al. [41]	1	+ve	Many cells were positive for CD68.
Fonseca et al. [24]	19	+ve	CD68 marker was easily identified in all cases.
Current study	21	+ve	

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