Strawberry Gingivitis of Wegener’s Granulomatosis: A Clinico-pathological and Immunohistochemical Case Study with Review of Literature

Bacem AE Ottoman1*

Received 2 March 2015; Published online 25 April 2015

© The author(s) 2015. Published with open access

Abstract

This paper discusses strawberry gingivitis of Wegener granulomatosis clinically, histologically and immunohistochemically. The reported case is compared to similar cases in the medical literature. Strawberry gingivitis is neither initially indicative for WG nor pathognomonic for it because it is evident in other fungal and carcinomatous conditions. However, this oral lesion, if clinically evident, can establish abundant information about the nature of the disease. The tested immunohistochemical markers (IHCMs) that are used in this study are Cathepsin K, and Myeloblastin (PR3). These two IHCMs proved to be strongly positive in WG. This histochemical profile can be useful in establishing the diagnosis of WG from a small friable gingival tissue. Accordingly, this paper describes and recommends a suggested protocol of diagnosing Wegener’s granulomatosis from a gingival lesional tissue. The superiority of this protocol banks mainly on simplicity, availability and fidelity of the involved procedures. Still, testing the gingival tissue would be informative and useful, unlike Anti-neutrophil cytoplasmic antibody (ANCA) test, if “strawberry gingivitis” was a manifestation of either fungal or carcinomatous conditions.

Keywords: strawberry gingivitis; Wegener’s granulomatosis; Oral manifestations; PR3 IHC expression; Cathepsin K; diagnostic protocol for WG.

1. Introduction

Wegener’s granulomatosis (WG), Wegener -Klinger syndrome or currently granulomatosis with polyangitis, is defined by Regezi et al. (2007) as an uncommon immune-based inflammatory necrotizing vasculitis with idiopathic etiology. It was described by McBride in 1897 as a midfacial granuloma syndrome, followed by Heinz Klinger, a Berlin pathologist, in 1932. However, the complete picture was completed in 1936’s “On Generalized Septic Vessel Diseases” and 1939’s “On a Peculiar Rhinogenic Granuloma with particular involvement of the arterial system and the kidney” by

*Corresponding e-mail: Bacemottoman@gmail.com
1 Maxillofacial surgery and diagnosis, Cairo University, Egypt.
Friedrich Wegener. Wegener has described post-mortem studies of two patients who died of disseminated vasculitis. In 1948, Sven Johnson, a Swedish pathologist, recognized Wegener’s granulomatosis as a distinct entity, separating it from periarteritis nodosa. Godman and Churg (1954) reviewed 22 cases of Wegener’s granulomatosis from published reports, adding seven new cases to explore the nature of this disease. In 1990, the American College of Rheumatology (ACR) has specified certain criteria for the classification of Wegener’s granulomatosis. In this classification, 4 criteria were selected: abnormal urinary sediment, abnormal findings on chest radiograph, ulcerative oral mucosa or nasal discharge, and granulomatous inflammation on biopsy. The presence of 2 or more of these 4 criteria was associated with a sensitivity of 88.2% and a specificity of 92.0% (Leavitt et al 1990).

WG usually presents as a triad of upper respiratory tract, lung, and renal involvement, mostly focal necrotizing glomerulonephritis. Langford and Fauci (2001) confirmed that diagnosing WG is based on clinical findings and positive anti-neutrophil cytoplasmic antibody against proteinase 3 (cANCA-PR3) serology. Strawberry gingivitis, if present, is very suggestive of the disease. It is either manifested initially or later in the course of the disease. More interestingly, strawberry mucosa might be localized intra-orally or extend to multiple mucosal organs (Bhatt and Hall, 2009; Steward et al., 2007; Siar et al., 2001; Cohen et al., 1990; Manchanda et al., 2003; Shiboski et al., 2002). This paper tackles studies strawberry gingivitis in an 11-year-old boy, diagnosed with indolent WG. Langford. (2003) enumerated diverse treatment options for WG including glucocorticoids, cyclophosphamide, methotrexate, azathioprine, mycophenolate, mofetil, cyclosporin, deoxyspergualin, intravenous immunoglobulin, sulfamethoxazole, and rituximab.

2. A Case Study

An 11-years-old boy presented to the Department of oral and maxillofacial surgery and diagnosis, Shubra hospital (Ministry of Health, Egypt) with a chief complaint of easily bleeding gums. His father reported a surgical history of esophagectomy in 2006 that delivered the kid to a gavage (enteral feeding or tube feeding using the so-called ryles for nutritional supplementation). Unfortunately, the father refused to give any further details. He asked for treating only the oral condition with paying no attention to diagnosing the underlying cause because he could not tolerate any further medical investigations. Intraoral examinations revealed necrotizing gingivae, recapitulates the appearance of over-ripen strawberry, and excessive salivation (Figure 1 A &B).

The father was educated about the tentative diagnosis of Wegener’s granulomatosis: a diagnosis that accounts for any past upper respiratory granulomatous condition and includes many renal and pulmonary signs and symptoms. After some time, he allowed us to get an incisional biopsy. Under local anesthesia (1.8 ml of 2% Lidocaine), the harvested gingivae were placed immediately into 10% formalin and submitted for microscopic examination. The exposed surface was packed using zinc oxide and eugenol paste. Dexamethasone was also described.

From the block of the represented case, serial sections from formalin-fixed, paraffin-embedded specimen block of 4 μm thickness were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Endogenous peroxidase activity was blocked by immersing the sections in 3% H₂O₂ with methanol for 30 min. For antigen retrieval, sections were boiled in 10 mmol/L citrate buffer (pH 6.0) for 15 minutes in a pressure cooker. After treatment with protein block
serum at room temperature, sections were covered with primary antibodies; Cathepsin K (3F9, Abcam, 1:300), and Myeloblastin (PR3) (Polyclonal, Dako, 1:500). Immunoreaction was performed using the labeled streptavidin-biotin method and overnight incubation. For all antibodies, negativity or positivity was evaluated.

Histological examination revealed an overlying oral epithelium that is hyperplastic and detached with areas of acanthosis and edema. The edematous connective tissue manifested a heavy neutrophilic and lymphocytic infiltrate with focal areas of fibrinoid necrosis and vasculitis. The histologic picture was necrotizing gingivitis that is, along with the clinical data, suggestive of Wegener’s granulomatosis for further clinical correlation and immunohistochemical study. (Figure 2 A &B). Immunohistochemically, Cathepsin K and PR3 were strongly positive (Figures 3 & 4).

**Fig. 1.** Clinico-pathological picture of strawberry gingivitis of all oral quadrants. (Left) The labial and buccal gingivae (right) the palatal gingiva: hemorrhagic friable gingivae with dark red bumpy surface that recapitulates the appearance of over-ripen strawberry.

**Fig. 2.** (a) Incisional gingival biopsy (hematoxylin-eosin, original magnifications 4x). The gingival tissue shows edematous detached overlying epithilium and heavy stromal inflammatory infiltrates in the connective tissue. (b) hematoxylin-eosin photomicrograph (original magnifications 20x) the dense polymorphous inflammatory cell infiltrates composed of mature lymphocytes, plasma cells, histiocytes, eosinophils and abundant neutrophils.
Fig. 3. Photomicrograph showing expression of Cathepsin K (Original magnification 40×). It shows positive expression of Cathepsin-K by the entrapped granulomas.

Fig. 4. Photomicrograph showing expression of PR3 (Original magnification 100×). Strong positivity for PR-3 supports the heavy neutrophilic induced vasculitis.

3. Discussion

Wegener’s granulomatosis, also known as granulomatosis polyangiitis, is an uncommon immune-based inflammatory necrotizing vasculitis with no certain etiology proven. Neville et al. (2009) Infectious trigger, autoimmunity, genetic predisposition and radiations are suspected. Jagiello et al. (2004) identified two candidate genes on chromosome 6, RXRB and butyrophilin-like 2 (BTNL2) located on chromosome 6p21.3, in the vicinity or even in the respective WG-associated region. In 6% to 13% of cases, painful cobblestone hyperplastic, granular alterations of oral gingiva “strawberry gingivitis” should be evident. The clinical differential diagnosis, according to Regezi

(2007), is broad and includes fungal disease, squamous cell carcinoma, lymphoma, infectious granulomatous disease, Langerhans cell disease, metastasis, and other rare conditions. The onset of strawberry gingivitis aside, Cohen et al (1990) and Neville et al (2009) confirm that strawberry gingivitis is always a caveat for serious conditions; thence, WG and cancerous diseases come atop. Typically, the triad of upper respiratory tract, lung, and renal involvement is seen in WG.

Ominously, other reported clinical manifestations are too numerous. Hoffman et al. (1999) and Tarabishy (2010) have enlisted numerous manifestations. Upper respiratory signs and symptoms include ear epistaxis, sinonasal dryness, crusts, obstruction, sinonasal destruction, subglottic stenosis, hearing loss, tinnitus and vertigo. Lower respiratory manifestations comprise parenchymal nodules, endobronchial lesions, pulmonary infiltrates, and pulmonary hemorrhage or embolism. Other signs and symptoms involve crescentic glomerulonephritis, renal mass lesions, myalgias, arthralgias, arthritis, orbital pseudotumor, conjunctivitis, episcleritis, scleritis uveitis, retinitis, nasolacrimal obstruction, purpura, Sensory neuropathy, mononeuritis, multiplex pachymeningitis, pericarditis, myocarditis, and cor pulmonale.

Presence of ANCA in the plasma of approximately 90% of WG patients reflects autoimmune background of the disease. According to Specks (2002), in WG patients ANCAs are mostly directed against proteinase 3 (PR3), presented in primary azurophil granules of polymorph nuclear neutrophils (PMN) and lysosomes of monocytes. After cytokine priming of PMN, PR3 translocates to the cell surface where ANCAs can bind and activate PMN resulting in a respiratory burst and release of proteolytic enzymes. Van der Geld et al. (2001) and Agarwal et al. (2004) have identified the ANCA as a pathogenic factor in inflammatory processes that underlies necrotizing vasculitis. The sensitivity of c-ANCA in active Wegener’s disease is up to 91% and a specificity of 99%. Accordingly, diagnosticians, who highly suspect WG, test for seropositivity of ANCA. WG is a multifaceted disease whose diagnosis requires a certain stroke of luck: to observe a strawberry gingiva or to move the diagnosis of WG ahead of the basic list of differential diagnoses.

Histologically, the basic pathological process is granulomatous, with heavy neutrophilic necrosis and nuclear dust (leukocytoblastic vasculitis). Necrosis and multinucleated giant cells may be seen in granulomatous areas. Affected small vessels show a mononuclear infiltrate within their walls in the presence of fibrinoid necrosis. In gingival biopsies, the oral epithelium may be hyperplastic and edematous with areas of pseudoepitheliomatous hyperplasia and subepithelial microabscesses. Owing to the paucity of large vessels in many oral biopsies, the vasculitis may not be conspicuous. Moreover, the granulomatous changes may be sparse and entrapped. Thus, the use of immunohistochemical marker will be of great value (Neville et al., 2009; Regezi et al., 2007 and Steward et al., 2007)

In the azurophilic granules of neutrophils and peroxidase-positive lysosomes of monocytes, there are two target antigens; proteinase 3 and myeloperoxidase (MPO). Toward detecting them, two patterns of ANCA exist; c-ANCA and p-ANCA. The c-ANCA pattern indicates diffuse staining throughout the cytoplasm, and in most cases the antibodies responsible for this pattern are directed against PR3. The p-ANCA pattern indicates staining around the nucleus, and the antibody responsible for this pattern is usually against MPO. In WG, the seropositivity of c-ANCA is more reliable. However, the accuracy of the results is operator-dependent and varies according to the experience of the laboratory personnel interpreting ANCA immunofluorescence results (Hoffman &
Specks 1998). Since detecting PR3 is what matters, it is more rational to easily detect it in a friable gingival tissue.

The availability and seropositivity of cANCA has dissuaded diagnosticians from performing any other histological examinations which may require a major surgical intervention. This plea is not working in case of incising and harvesting a friable gingival tissue under local anesthesia: a procedure that any average practitioner can successfully perform. The obtained information from the blood test versus those that can be obtained from the microscopic sections should not be equivalent. The serologic testing for cANCA is useful, if positive, in classical Wegener's granulomatosis but neither in fungal nor sarcomatous nor canceromatous conditions. The histological findings are very useful in indubitably categorizing the pathological lesion (Neville et al. 2009). Ethically speaking, testing the patient for once is more advocated more than trying many tests if diagnosis was not luckily established.

On the level of immunohistochemical findings, Díaz and Willis (2000) have concluded that Cathepsin K, a cysteine proteinase, has aroused intense interest as the main effector in the digestion of extracellular matrix during bone resorption and granulomatous responses, is expressed by epithelioid and giant multinucleated cells allowing for detection of entrapped microgranulomatus areas. Cathepsin K is positive in the studied case, expressing the entrapped granulomas (fig. 3).

Characteristically, myeloblastin (PR3) or Wegener granulomatosis autoantigen is a polymorphonuclear leukocyte serine protease which plays a role in neutrophil response to inflammation. Part from being available for serology, Agarwal and Gogia (2004) and Csernok et al. (1990) have presented it as an immunohistochemical marker that traces neutrophilic infiltration and its induced vasculitis. In this study, PR3 was strongly expressed in WG (fig. 4).

4. Conclusion

Strawberry gingivitis biopsy, when combined with immunohistochemical investigations, is highly diagnostic of Wegener's Granulomatosis. Toward less invasive and more measureable results, PR3 is suggested to be used as immunohistochemical marker since its seropositivity is originally tagged. The represented case is meant to be just exemplary so is the studied case. The number of cases should not be a problem because the same principle is applied upon a different target; strawberry gingiva.

The procedures of the suggested protocol should run as follows:
1. Under local anesthesia (1.8 ml of 2% Lidocaine), the most friable intraoral lesional gingiva should be anesthetized and incised.
2. The exposed surface is packed using zinc oxide and eugenol paste or periodontal pack (dressing).
3. The incised biopsy is submitted to microscopic examination. If heavy neutrophilic and lymphocytic infiltrate with focal areas of fibrinoid necrosis and vasculitis can be observed, WG is highly suggested.
4. Section from the paraffin-embedded specimen block should be stained by IHCs; PR3 and Cathepsin-K as described earlier.
5. Positive expressions of both markers confirm the diagnosis of WG.
Acknowledgments

I would like to acknowledge my cousin Dr. Arwa M. Talaat for her vocational guidance and careful reviewing. My acknowledgment extends to my colleague Dr. Amany Khalifa, as well as the anonymous reviewers for their useful comments.

Competing interests: None.

Funding sources: None.

References


http://dx.doi.org/10.1016/j.bjoms.2009.02.012

http://dx.doi.org/10.1902/jop.1990.61.11.705


http://dx.doi.org/10.7326/0003-4819-116-6-488

http://dx.doi.org/10.1002/1529-0131(199809)41:9<1521::AID-ART2>3.0.CO;2-A

http://dx.doi.org/10.1007/s00439-004-1092-x


http://dx.doi.org/10.1186/ar771

http://dx.doi.org/10.1002/art.1780330807


