Review Article

Neutrophils defending the defenders

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Abstract

Neutrophils are the most abundant granulocytes which are involved in defense mechanism. As innate immune cells, they are first-line defenders and can perform different functions in the human body to maintain equilibrium. Neutrophils are the main leukocyte and their role in healthy oral cavity is to face pathological changes within oral environment. With regard to these, it has been observed that neutrophils are highly heterogeneous in their behavior. The aim of this review is to give an overview of the role of neutrophils in context of various physiological and pathological conditions.

Keywords: Host defense, neutrophil disorders, neutrophils, phagocytosis

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INTRODUCTION

Neutrophils also known as granulocytes are an essential component of the cellular innate system involved in killing bacteria and fungi. They play a major role in host defense by phagocytizing and digesting microorganisms.[1] Neutrophils, the most numerous of the mobile phagocytes, have long been recognized for our innate immunity against invading microorganisms.[1]

Neutrophils are generated in bone marrow and their production rate is boosted up to 10 folds whenever there is infection.[2] The blood and bone marrow form an abundant pool of cells and neutrophils are recruited to the site of infection or inflammation where they are stimulated by a cytokine.[3]

Though oral cavity is heavily colonized with microorganisms yet the infection occurs rarely due to active equilibrium between oral microbiota and host immune response. In this review, the authors aim to discuss the diverse roles of neutrophils in health and in disease, with a specific role in the oral cavity. Furthermore, we focus on the response of neutrophils to face pathological and physiological conditions in the human body.

NEUTROPHIL PRODUCTION IN BONE MARROW

Origin of neutrophils

Neutrophils are produced in the bone marrow. According to Athens,[3] bone marrow neutrophil population can be divided into three pools: the stem cell pool, the mitotic pool and the postmitotic pool. The stem cell pool consists of undifferentiated hematopoietic stem cells (HCSs), whereas the mitotic pool refers to committed granulocytic progenitor cells that are undergoing proliferation and differentiation. Finally, the postmitotic pool contains fully mature neutrophils which are available for release [Figure 1].

In the early development of neutrophil, there are some receptors which are helpful in maintaining the development

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of neutrophils. Hernandez *et al*.[4] mentioned that granulocyte colony-stimulating factor (G-CSF) receptors are maintained at their surface. CXC chemokine receptor 4, a G protein-coupled receptor, is also expressed at low levels on the cell surface of mature neutrophils.

HCSs in the bone marrow produce a large number of neutrophils. These cells differentiate into multipotent progenitor (MPP) cells that cannot self-renew themselves. MPPs then transform into lymphoid-primed multipotent progenitors, which differentiate into granulocyte–monocyte progenitors.[5]

These cells under influence of G-CSF follow the maturation process that includes the stages of promyelocyte, myelocyte, metamyelocyte, band cell and finally mature neutrophil.[6] To exit bone marrow, neutrophils migrate across the bone marrow endothelium through tight-fitting pores by a singular process of transcellular migration.[7]

STRUCTURE OF NEUTROPHILS

Neutrophil contains granules and secretory vesicles that store specific proteins relevant to their functions. Granules are of three types: namely primary (azurophilic) granules, secondary (specific) granules and tertiary (gelatinase) granules.

According to Amulic *et al*.,[8] azurophilic granules are the largest measuring approximately 0.3 μm in diameter. They are named because they take up the basic dye azure A and contain myeloperoxidase (MPO), an enzyme helpful in oxidative burst. The second class granules are smaller 0.1 μm, do not contain MPO and are characterized by the presence of glycogen lactoferrin.

Tertiary granules are also MPO negative, contain few antimicrobials and serve as storage location for number of metalloproteases. Finally, tertiary granules serve as a reservoir for a number of important membrane-bound molecules employed during neutrophil migration.[8]

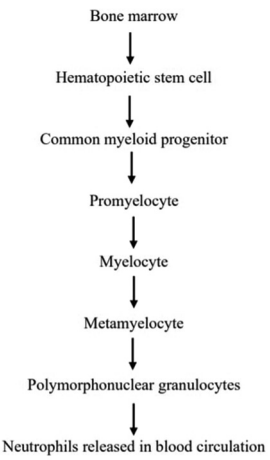

Figure 1: Production of neutrophils

MECHANISM OF ACTION

Leukocytes are recruited from blood into extravascular tissue and migrate to the site of infection and are activated to perform their functions. Leukocyte recruitment is a multistep process which comprises firstly loose attachment to and rolling on endothelium wall, secondly firm attachment to endothelium, thirdly margination through interendothelial spaces and finally migrate to the site of injury under influence of chemotactic agents [Figure 2].[1,5]

FUNCTIONS OF NEUTROPHIL

Neutrophil as oxidant

Neutrophils are potent and robust phagocytes. Song *et al*.[9] stated that whenever there is any type of infection or inflammation, neutrophil granules fuse with phagosome and release antimicrobial contents. At the same time, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated which results in the production of oxygen radicals, and this NADPH oxidase plays an important role in microbial killing.

Neutrophil and tissue repair

Neutrophils are a significant source of matrix metalloproteinases (MMPs), which are able to cleave matrix

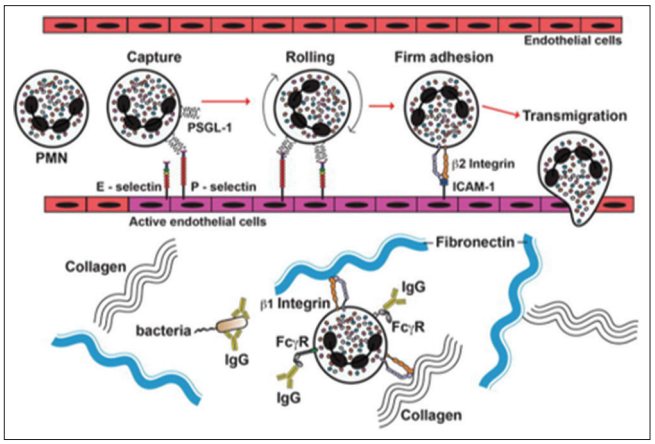

Figure 2: Neutrophils move to the site of inflammation via leukocyte adhesion cascade that includes activation of endothelial cells with upregulation of E- and P-selections. Neutrophils bind to these selections via glycoprotein ligands such as P-selection glycoprotein ligand-1 and begin rolling on endothelial cells. Next, neutrophils get stimulated by chemokines and activate β_1-integrin, which bind to their corresponding ligands such as intercellular adhesion molecule-1. Integrin binding induces firm adhesion and transmigration of neutrophil in tissues. Once in tissues, neutrophils reach to affected site using adhesion molecule β_1-integrin to extracellular matrix, such as collagen and fibronectin. Antibodies (immunoglobulin G) bind to microorganism and are in turn recognized by FcY (Fc gamma) receptor on the membrane of microorganism[5]

such as elastase, collagen and degrade immune receptors.[7] Hence, this cleavage helps to stimulate the immune system and send a positive feedback to tissue degradation.

The inflammatory response after tissue injury is a dynamic process composed of sequential steps and aimed at restoring tissue architecture and function. Three possible strategies which are adopted by neutrophils to promote tissue repair include. (1) Neutrophils can clear necrotic cellular debris, (2) Neutrophils release effectors that promote angiogenesis and regeneration, (3) Phagocytosis of apoptotic neutrophils results in release of anti inflammatory and reparative cytokines.[10,11]

Neutrophils in dentogingival sulcus and saliva

It is interesting to note that the main entry of leukocytes into oral cavity is gingival sulcus. The majority of these cells have phagocytic and killing capacity. Therefore, they constitute a major protective mechanism against extension of plaque into gingival sulcus.[12]

In dentogingival sulcus, bacteria are always present, and migration of polymorphonuclear neutrophils (PMNs) in the gingival crevice is continuous. Neutrophils are viable and functional in the gingival crevice; According to Grant PR[13] neutrophils form a "leukocyte wall" between the plaque and the epithelium tissues. . The leukocyte wall has proteolytic, phagocytic and antibacterial features.

The main role of PMNs, however, is phagocytosis, and the PMNs in the gingival tissues are the main controllers of the microbial ecology within the gingival crevice. The cross talk between neutrophils and immune cells is constant. Neutrophils have surface receptors for both complement and immunoglobulin, and the interaction with opsonized bacteria leads to phagocytosis of the bacteria.[14]

Neutrophil interface with microorganisms

Leukocytes are infiltrating junctional epithelium mainly, but other cells such as lymphocytes and monocytes also predominate within the subadjacent connective tissue.

The leukocyte wall may function as a secretory and as a digestive organ whereas crevicular neutrophils help in partial degranulation and ingestion of bacteria.[3]

DISORDERS ASSOCIATED WITH NEUTROPHILS

Neutropenia

Neutropenia is a reduction in circulating neutrophils. Reference ranges vary with age; infants have lower neutrophil count than older individuals.

The decrease in neutrophil count may hamper the inflammatory response, thus reducing the ability to localize the infection and permit rapid disintegration. The most common causes of infections in individuals with chronic neutropenia are mainly endogenous flora: *Staphylococcus* species, *Klebsiella* species and *Aspergillus* species.[15]

Cyclic neutropenia is a rare disease characterized by cyclic depression of the peripheral blood PMNs count at a 21-day interval.[16-18] Neutropenia predisposes to recurrent oral ulceration and to respiratory or cutaneous bacterial infections.[19] Oral ulceration and destructive periodontal disease have been reported in most of the recorded cases and are frequently the major manifestation in about 20% of patients with cyclic neutropenia.[20]

Severe congenital neutropenia

Children with severe congenital neutropenia are characterized by the early onset of life-threatening infections, severe persistent neutropenia and maturation arrest at the myelocyte/promyelocyte stage.

The condition usually presents in the first few months of life with life-threatening infections such as septicemia, meningitis and respiratory infections. These infections are caused by *Staphylococcus aureus, Escherichia coli* and *Pseudomonas* species.[15]

Investigations reveal absolute neutropenia, which may be present from the 1st day of life, with neutrophil counts persistently below 0.2×10^8/L of blood.[21]

The typical symptoms, which appear since the 1st day of life, are abscesses located on various parts of the body: ear, cutis, lung and oral cavity.[22] These abscesses are due to an almost total disimmunity typical of neutropenia.

Chronic granulomatous disorder

It is a heterogeneous group of disorders characterized by defective generation of a respiratory burst in human phagocytes.[9] Chronic granulomatous disorder (CGD) is a severe congenital disorder of neutrophil function which is to be recognized.[12] The resultant defect is an inability to generate superoxide and hence an inability to contain certain infectious pathogens. The disease manifests as repeated, severe bacterial and fungal infections resulting in the formation of inflammatory granulomas.[9]

The most commonly encountered pathogens are *S. aureus, Aspergillus* species and enteric Gram-negative bacteria.[15]

Carriers of autosomal recessive forms of CGD are asymptomatic. Approximately half of the X-linked carriers

suffer from recurrent stomatitis and/or gingivitis and about 25% develop discoid lupus erythematosus on sun exposure. A very small proportion of X-linked CGD carriers may suffer from increased, although mild, infections.[15]

Leukocyte adhesion deficiency

Neutrophil migration from bloodstream through endothelium is mediated by adhesion molecules and ligands.

The term leukocyte adhesion deficiency (LAD) was first described by Anderson and Springer *et al*.[23] for a condition caused by a defect of the β_2-integrins, heterodimers of α and β subunits called CD11 and CD18, respectively.[15]

LAD type I (LAD I) is due to either complete or partial lack of CD18, the common subunit of the β_2-integrin.[1] Infections mainly affect the skin and mucous membranes, infection sites often undergo necrosis, but characteristically, there is no pus, even though there is pronounced peripheral blood neutrophilia.[15]

Recurrent infections such as ulcerative stomatitis, gingivitis and periodontitis are seen. The diagnosis can be confirmed by flow metric analysis of peripheral blood neutrophils using monoclonal antibodies for CD11 or CD18.[24]

LAD II is caused by a congenital disorder of fucosylation of ligands for selectins and other glycoconjugates.[11]

These integrins are protein complexes that are stored within the neutrophil granules, Integrins are the protein complexes which are stored within the neutrophil granules, when activated they are released on neutrophil membrane so that leucocyte can adhere on endothelium wall.

It is associated with severe mental retardation and short stature and presents with frequent infections similar to the moderate-to-mild phenotype of LAD1.[15]

Specific granule deficiency

It is a rare disorder characterized by infections, particularly in skin and mucous membranes, and has the inability to perform full respiratory burst and have defective chemotaxis.[1]

The neutrophils lack the characteristic granule proteins of specific granules. The defect is not only restricted to granule proteins of neutrophils but also includes eosinophil granule proteins. Specific granule deficiency individuals are severely immunocompromised and develop frequent bacterial infections, including *Pseudomonas aeruginosa* and *S. aureus*.[25]

Chediak–Higashi syndrome

Chediak–Higashi syndrome (CHS) is an autosomal recessive disorder of granule-bearing cells. It is caused by mutations in the lysosomal trafficking regulator gene, present on the long arm of chromosome 1 (1q42.1–q42.2).[26]

There is uncontrolled granule fusion leading to defective granules in all granule-bearing cells including Schwann cells, melanosomes and neutrophils.

Children with CHS present in childhood with partial albinism (hypopigmentation of hair and eyes compared with other family members) and recurrent infections of the skin, mouth and respiratory tract.[27]

According to Karabel *et al*.,[28] there are two phases of the disease. The first is the stable phase, which is characterized by recurrent infections such as periodontitis and gingivitis due to neutropenia and/or neutrophil dysfunction and hypopigmentation of the hair, skin and eyes. It typically develops in infancy or early childhood in most patients with CHS. The second phase is the accelerated phase, which is characterized by persistent fever, hepatosplenomegaly, lymphadenopathy, pancytopenia and bone marrow infiltration and hemophagocytosis by histiocytes.

ROLE OF NEUTROPHIL IN PULPAL INFLAMMATION AND PERIAPICAL LESIONS

Infection-stimulated inflammatory process involves the dental pulp prior to affecting the periapical lesion. Early pulpal responses to pulp exposure and bacterial invasion and/or the diffusion of bacterial products through dentinal tubules include the influx of PMNs and monocytes.[29]

As the infection progresses, the cellular infiltrate becomes more intense and assumes a typical "mixed" character, consisting of T4+ (T-helper) and T8+ (T-cytotoxic/suppressor) T-cells, B-cells and plasma cells as specific elements, along with PMNs, monocytes and natural killer (NK) cells as nonspecific components.[29]

Many studies have described the inflammatory cell infiltrate in chronic periapical lesions in both human and nonhuman models. These reports demonstrate that, similar to the pulp, a mixed infiltrate consisting of T- and B-lymphocytes, PMNs, macrophages, plasma cells, NK cells, eosinophils and mast cells is present.[29]

Neutrophils are an absolutely essential part of the innate immune system, playing a necessary role in the control of infectious diseases, but more recently are also being viewed as important players in tissue repair. Neutrophils

are capable to act on infection through phagocytosis and release neutrophil extracellular traps.[30]

Inflammation may also lead to the generation of neutrophil subpopulation, some have immunosuppressive properties and some have shown pro-inflammatory functions.[28]

NEUTROPHILS AND ORAL CANCER

PMN-derived suppressor cells are the main neutrophil subpopulation in blood of cancer patients.[31] They display pro- or antitumor functions within the circulation and tumor tissue of cancer patients.

Jaiswal *et al*.[32] mentioned that the development of oral squamous cell carcinoma or any malignancy is associated with a complex biological cross talk between tumor cells, stromal cells and host inflammatory cells. The host defense barrier is generally carried out by the neutrophils, macrophages and lymphocytes.

Tumor-associated neutrophils can promote tumor progression and metastasis through different mechanisms, including the activation of cytokines, integrin binding, reactive oxygen species formation, secretion of neutrophil elastase (NE) and proteases. The role of NE in cancer has largely been attributed to its ability to degrade extracellular matrix proteins and cause activation of MMP-2, thereby promoting invasion and metastasis.[32]

NEUTROPHILS AND ORAL HEALTH

In the mouth, the barriers to prevent microbial invasion are the oral mucosal surfaces that cover the jaws, the cheeks, the tongue, etc., The oral mucosa is considered to be one of the main ecological habitats of the human body.[33]

A particularly vulnerable site in the oral cavity is the epithelium of the gingival crevices surrounding the teeth. While this location may function as a port of exit for the oral PMNs to enter the oral cavity, microorganisms can also use the same site as a port of entry. This site is therefore under continuous microbial exposure, which needs to be critically guarded. It seems only logical that PMNs are needed to operate at the gingival barrier in health.

Rijkschroeff *et al*.[34] mentioned that since these sites are additionally under continuous stress due to oral functions such as speech, repetitive mechanical force from chewing and hygiene habits, the oral cavity and the oral mucosal barrier are therefore distinct from other barrier sites in the human body.

The delicate balance in the mouth between the oral microbiome and the innate immunity, including an important role for neutrophils, is best reflected when equilibrium is lost, leading to progressive inflammation. As an example, neutrophils lacking NADPH oxidase activity are unable to provide a respiratory burst, resulting in the inability to kill microorganisms effectively. As a consequence, patients lacking the respiratory burst capability are profoundly immunodeficient and present with frequent acute and chronic oral infections.[33] More research is required to further understand the interrelations between PMNs and microbiota within the healthy oral ecosystem and to assess the changes occurring within the ecosystem.

ROLE OF NEUTROPHILS IN COVID-19

In viral infections of upper respiratory tract, the role of neutrophils is to provide early antiviral defence which is achieved by degranulation and lysis. This process has cytotoxic effect during severe pneumonia and coronavirus infection.[35]

COVID 19 is commonly associated with hyperinflammation that drives lung or multiorgan injury. The immunopathological mechanisms that cause excessive inflammation are under investigation and constantly updated.[36]

In the current COVID-19 literature, an increased peripheral neutrophil-to-lymphocyte ratio is observed in severe cases and is probably related to unfavorable prognosis.[33]

According to a study by Didangelos A,[36] recently neutrophil infiltration was also noted in the lung tissue of autopsied COVID-19 patients. Since neutrophilia predicts poor outcomes in patients with COVID-19, the change in neutrophil counts in peripheral blood or tissues is also closely related to pathological injury in COVID-19 patients. The dynamics of neutrophil counts in COVID 19 patients during hospitalization exhibited the same trend as the corresponding lung injury.[36]

The mechanisms behind this are not understood, and not much is thought regarding neutrophil activity in SARS-CoV-2-infected lungs. COVID-19 lung damage in some patients might involve dysregulated neutrophil activity. The possible role of neutrophils in COVID-19 inflammation must be studied further.

CONCLUSION

The human body has many ways to protect itself; some are simply physical barriers like the skin. Chemical barrier is provided by blood proteins which destroys

microorganisms and foreign substances through effective defence mechanism.

Neutrophils are a hallmark of an inflammatory response and the most mobile phagocytes required for innate immunity against microorganisms. Finally, improved understanding of metabolic functions in neutrophil population migrating to infection or inflammatory sites and their survival could be helpful in understanding its function in various disorders.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Häger M, Cowland JB, Borregaard N. Neutrophil granules in health and disease. J Intern Med 2010;268:25-34.
2. Liew PX, Kubes P. The neutrophil's role during health and disease. Physiol Rev 2019;99:1223-48.
3. Athens JW. Blood: Leukocytes. Annu Rev Physiol 1963;25:195-212.
4. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, *et al*. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet 2013;34:663-72.
5. Kourtzelis I, Mitroulis I, von Renesse J, Hajishengallis G, Chavakis T. From leukocyte recruitment to resolution of inflammation: The cardinal role of integrins. J Leukoc Biol 2017;102:677-83.
6. von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. J Immunol 2008;181:5183-8.
7. Burdon PC, Martin C, Rankin SM. Migration across the sinusoidal endothelium regulates neutrophil mobilization in response to ELR+CXC chemokines. Br J Haematol 2008;142:100-8.
8. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: From mechanisms to disease. Annu Rev Immunol 2012;30:459-89.
9. Song E, Jaishankar GB, Saleh H, Jithpratuck W, Sahni R, Krishnaswamy G. Chronic granulomatous disease: A review of the infectious and inflammatory complications. Clin Mol Allergy 2011;9:10-5.
10. Weathington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, *et al*. A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. Nat Med 2006;82:1038-361.
11. Wang J. Neutrophils in tissue injury and repair. Cell Tissue Res 2018;371:531-9.
12. Miyasaki KT. The neutrophil: Mechanism of controlling periodontal bacteria. J Periodontol 1991;15:516-23.
13. Garant PR. Plaque-neutrophil interaction in monoinfected rats as visualized by transmission electron microscopy. J Periodontol 1976;47:132-8.
14. Pöllänen MT, Laine MA, Ihalin R, Uitto VJ. Host-bacteria crosstalk at the dentogingival junction. Int J Dent 2012;2012:821383.
15. Lakshman R, Finn A. Neutrophil disorders and their management. J Clin Pathol 2001;54:7-19.
16. Rutledge BH, Hansen-Pruss OC, Thayer WS. Recurrent agranulocytosis. Bull Johns Hopkins Hosp 1930;46:369.
17. Morley AA, Carew JP, Baikie AG. Familial cyclical neutropenia. Br J Haematol 1967;13:719-38.
18. Guerry D 4th, Dale DC, Omine M, Perry S, Wolff SM. Periodic hematopoiesis in human cyclic neutropenia. J Clin Invest 1973;52:3220-30.
19. Page AR, Good RA. Studies on cyclic neutropenia. A clinical and experimental investigation. J Dis Child 1957;94:623.
20. Cohen DW, Morris AL. Periodontal manifestations of cyclic neutropenia. J Periodontol 1961;32:159.
21. Kostman R. Infantile genetic agranulocytosis. A review with presentation of ten new cases. Acta Paediatr Scand 1975;64:362-8.
22. Defraia E, Marinelli A. Oral manifestations of congenital neutropenia or Kostmann syndrome. J Clin Pediatr Dent 2001;26:99-102.
23. Marlin SD, Morton CC, Anderson DC, Springer TA. LFA-1 immunodeficiency disease. Definition of the genetic defect and chromosomal mapping of alpha and beta subunits of the lymphocyte function-associated antigen 1 (LFA-1) by complementation in hybrid cells. J Exp Med 1986;164:855-67.
24. Finn A, Rebuck N. Measurement of adhesion molecule expression on neutrophils and fixation. J Immunol Methods 1994;171:267-8.
25. Rosenberg HF, Gallin JI. Neutrophil-specific granule deficiency includes eosinophils. Blood 1993;82:268-73.
26. Barrat FJ, Auloge L, Pastural E, Lagelouse RD, Vilmer E, Cant AJ, *et al*. Genetic and physical mapping of the Chediak-Higashi syndrome on chromosome 1q42-43. Am J Hum Genet 1996;59:625-32.
27. Spritz RA. Chediak-Higashi syndrome. In: Ochs HD, Smith CIE, Puck JM, editors. Primary Immunodeficiency Diseases – A molecular and Genetic Approach. New York: Oxford University Press; 1999. p. 389-96.
28. Karabel M, Kelekçi S, Sen V, Karabel D, Aliosmanoğlu C, Söker M. A rare cause of recurrent oral lesions: Chediak-higashi syndrome. Turk J Haematol 2014;31:313-4.
29. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 2013;41:211-5.
30. Castanheira FV, Kubes P. Neutrophils and NETs in modulating acute and chronic inflammation. Blood 2019;133:20.
31. Roig CS, Fridlender ZG. Neutrophil diversity in health and disease. Trends Immunol 2019;40:7.
32. Jaiswal P, Kheur S, Mahajan P, Raj T, Reddy M, Mandalli R, *et al*. Assessing the potential role of neutrophil elastase as a prognostic indicator in oral squamous cell carcinoma. Forum Clin Oncol 2019;10:34-8.
33. Rijkschroeff P, Loos BG, Nicu EA. Oral polymorphonuclear neutrophil contributes to oral health. Curr Oral Health Rep 2018;5:211-20.
34. Rijkschroeff P, Loos BG, Nicu EA. Impaired polymorphonuclear neutrophils in the oral cavity of edentulous individuals. Eur J Oral Sci 2017;125:371-8.
35. Wang J, Li Q, Yin Y, Zhang Y, Cao Y, Lin X, *et al*. Excessive neutrophils and neutrophil extracellular traps in COVID-19. Front Immunol 2020;11:2063.
36. Didangelos A. COVID-19 hyperinflammation: What about neutrophils? mSphere 2020;5:3.