Eosinophilic Toto Bodies

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ABSTRACT

Aim: This review aims to discuss the structure, origin of toto bodies and demonstrate the possible theories responsible for its formation.

Background: The eosinophilic Toto bodies are characterized by homogeneous or irregular masses varying in size and number. These unusual bodies are found in the top most layers of oral epithelium and derive its name “mucopolysaccharide keratin dystrophy” based on the nature of its composition. The incidence and severity of toto bodies have been correlated with the amount of the inflammatory response present in the connective tissue. These bodies have various theories of origin like fibrin, keratin, mucins, and glycoproteins.

Review results: To demonstrate the nature of these eosinophilic bodies; two cases—pyogenic granuloma and oral squamous cell carcinoma were studied using special stains. It was observed that Toto bodies were positive for periodic acid-Schiff (PAS), modified Papanicolaou (Pap), Masson’s trichrome and combined PAS–PAP staining techniques. However, staining with Alcian blue, Congo red, mucicarmine and toluidine blue were negative for Toto bodies.

Conclusion: It may be concluded that Toto bodies can be best identified using Masson’s trichrome stain followed by combined PAS–PAP, PAS, and PAP which characterizes them mainly as glycoproteins and keratins.

Clinical significance: The presence of Toto bodies is indicative of inflammatory reaction, and their differential staining in the positive stains depends on the nature and severity of inflammation present in a lesion.

Keywords: Eosinophilic bodies, Inflammation, Toto bodies.

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INTRODUCTION

Toto bodies, also known as keratin mucopolysaccharide dystrophy are the eosinophilic pools of homogeneous masses present in the superficial spinous layer of epithelium. Various terminologies have been designated for Toto bodies such as “keratin pooling” and “keratin-like material”.¹

Toto bodies usually occur in small and large beaded coalescent masses that show variation in metachromasia. They occur in focal areas of superficial epithelium and are observed in mucocoeles, papillomas or redundant tissue with dentures. The present review focuses on the possible theories of origin, microscopic features and the special stains used for demonstration of Toto bodies.²

Light Microscopic Structure of Toto Bodies

Toto bodies are irregular eosinophilic bodies seen in varying sizes that range from small deposits intercellularly with a beaded linear appearance to large masses with a scalloped and convoluted border. These larger bodies are often discrete and separate although they are in close proximity with similar bodies. When these materials are viewed in higher magnification, they appear homogenous with focal vacuolated areas (Fig. 1A). Pyknotic nuclei can be observed with a thin rim of cytoplasm associated with this material (Fig. 1B). The intensity of staining of the individual globules is marked above the prickle cell layer.²

Ultrastructure of Toto Bodies

The keratin pools observed in the paraffin-embedded specimens have been initially reported by Archard et al.²

Fig. 1A: Photomicrographs of pyogenic granuloma showing vacuolated spaces in Pap stain (black arrows)

Fig. 1B: Extremely thin, compressed pyknotic nuclei around the pool of the eosinophilic material in PAS stain (yellow arrows) (X400)

and Chen et al.³ under electron microscopy to be composed of electron-dense amorphous material with numerous vacuoles or linear bands of fibrin-like fibers. The pools consist of scalloped border bound by a clear or slightly granular zone that is demarcated by a thin membrane of the neighboring cell. Cytoplasmic processes of superficial, parakeratinized epithelial cells can be appreciated between the pools in which tonofilaments are abundant with nuclei. The cells consist of small, cytoplasmic, electron-dense droplets that can be probably calcific in nature. They are associated with some fine tonofilaments that represent transitional stage in formation. A thin compressible nucleus is also seen in the cell. The cytoplasmic processes are connected to each other by desmosomes. The plasma membranes of the cells appear smooth, corrugated or villus-like projections. On closer inspection, bundles of tonofilaments that are associated with a disrupted desmosomal complex can be appreciated. The electron-lucent material present in the eosinophilic bodies is crystalline in appearance and can also be seen lying free in clusters within the cytoplasm.^{2,3}

Associated Lesions

The presence of “keratin pools” is generally found in association with oral inflammatory and other mucosal lesions where there is hyperplasia of the surface epithelium showing mucopolysaccharide dystrophy. Epulis fissuratum, irritational fibroma, pyogenic granuloma, peripheral giant cell granuloma, mucocoeles, and inflammatory hyperplastic gingivitis are few reactive lesions with which Toto bodies may be associated.¹⁻⁴ Archard et al. in 1970 stated that the presence of keratin pools can be expected in over 40% of specimens of epulis fissuratum when searched in depth.² In a previous study done by Buchner et al., it was found that the highest percentage of cases with eosino-

philic bodies was present in pyogenic granuloma followed by inflammatory hyperplastic gingivitis, peripheral giant cell granuloma, epulis fissuratum, and irritation fibroma. They have also found positivity of toto bodies in neoplasms.⁴ Hanusova et al. have also observed these toto bodies or keratin pools in the cutaneous lesions.²

Origin of Toto Bodies with Histochemical Composition

Various hypotheses have been proposed for the origin of toto bodies. According to Buchner et al., blood plasma cells or keratin-like material have been postulated as the origin for these bodies. These eosinophilic bodies represent a filtrate from the blood vessels resembling inflammatory exudate which is dependent on the intensity of the inflammatory reaction. Histochemically, the presence of –SH and –SS groups suggests a similarity to keratin. Therefore, the term keratin-like material for these masses has been suggested.^{1,5}

Chen et al. have found these bodies to be situated extracellularly in the dilated intercellular spaces of inflammatory lesions. Due to degeneration, the superficial cells of the epithelium are weakened, cytoplasmic degradation occurs and plasma membranes become thick resulting in reduced permeability to macromolecules. This also prevents the outlet of tonofilaments or keratohyaline granules into the intercellular spaces. Therefore, the authors have concluded that toto bodies are made of glycoproteins and mucopolysaccharide of normal intercellular substances and exudates of plasma fluid.³ Similarly, Sah et al. described toto bodies as homogeneous dystrophic complexes of acid and neutral mucopolysaccharide with keratin and justified its name, “Mucopolysaccharide Keratin Dystrophy.”¹

In view of various components that Toto bodies are made of, special stains (Table 1) have been employed to demonstrate the Toto bodies in two cases—reactive lesion and malignant neoplasm with secondary inflammation. Pyogenic granuloma was chosen among the reactive group of lesions as they possess common histogenesis for toto bodies. Well-differentiated squamous cell carcinoma represented the neoplastic group that showed Toto bodies as a secondary inflammatory change. The Toto bodies were demonstrated using special stains like periodic acid Schiff, modified Papanicolaou (PAP), Masson’s trichrome, combined PAS-PAP, Alcian blue, Congo red, mucicarmine and toluidine blue. The preference for these special stains was mainly due to ease of availability and being economical.

DISCUSSION

The present review demonstrates two cases with toto bodies—pyogenic granuloma and well differentiated

Table 1: Comparison of Special stains for the demonstration of Toto bodies

Sl. No.	Staining method	Indication	Results	Color of Toto bodies
1	Periodic acid Schiff	Glycoprotein	+	Magenta
2	Papanicolaou stain	Keratin	+	Orange to shades of pink
3	Periodic acid Schiff–Papanicolaou stain	Glycoprotein and keratin	+	Bright red
4	Masson's trichrome	Keratin/fibrin	+	Grey pink
5	Alcian blue	Protein	–	Light pink
6	Mucicarmine	Mucin	–	Light pink
7	Congo red	Amyloid	–	Dull red
8	Toluidine blue	Mucin, amyloid	–	No uptake of stain

squamous cell carcinoma that showed irregular eosinophilic bodies above the spinous cell layers of oral epithelium amidst dilated intercellular spaces. Both the cases supported the hypothesis of origin of Toto bodies by Buchner et al. that the eosinophilic material is caused due to collection of plasma that has permeated from the underlying infected stroma and is mainly made of inflammatory exudates.⁵ Therefore, its presence and differences in the intensity of staining is found to be highest in the lesions characterized by heavy inflammatory infiltrate.^{3,5}

Masson's trichrome as the name implies uses three dyes that selectively stain muscle, collagen/keratin, fibers, fibrin, and erythrocytes.⁶ The current cases were positive for Masson's trichrome stain with the best contrast showing varied colors of toto bodies ranging from grayish blue to magenta (Figs 2A and B). There are two possibilities for a positive reaction:

- *Fibrin/plasma:* Besides the cytoplasmic degeneration taking place, the plasma membrane of the superficial epithelial cells and the parakeratinized cells also become thickened. Such a plasma membrane is less permissible to the macromolecules of the fibrin leading to collection of fibrin and plasma. The variation in staining quality is dependent on the degree of keratinization and the amount of fibrin content during the progression of the inflammatory lesions and malignancy.³
- The presence of keratin-like material is justified by its superficial location and abnormal maturation that is triggered by inflammation.⁵

Among the various stains, periodic acid-Schiff (PAS) staining showed positivity for toto bodies in both the cases (Figs 2C and D). PAS is the most widely used technique for the demonstration of carbohydrates, glycogen and certain glycoproteins.⁷ The PAS staining technique involves the reaction of free aldehyde groups with carbohydrates and Schiff reagent to form a bright red magenta as the final end product.⁶ Glycoproteins and mucoproteins are the normal components of the blood plasma. Most of the carbohydrates and proteins present in the Toto bodies may represent those derived from the exuded plasma and shows positivity for PAS.³

Keratins are most abundant cellular proteins that constitute the major component of the cytoskeleton of all epithelia.⁷ Modified PAP stain is another special staining procedure that differentiates cells at different stages of differentiation due to the presence of their low molecular weight keratin filaments.⁸ The main component that stains keratin is orange G6 which showed positivity in the present cases (Figs 2E and F). Kumar reported that different nature of keratin molecules within the cell probably explained the difference in staining quality of modified Papanicolaou stain.⁸ Percy et al. reported the application of the Pap stain for the demonstration of keratin.⁹

Combined Periodic acid Schiff-Papanicolaou (PAS-Pap) technique was devised that enables confirmation of glycoproteins and keratin. In the present cases, the effectiveness and sensitivity were more compatible and accurate when the tissue sections were stained with combined PAS-Pap than when used alone for the identification of toto bodies (Figs 2G and H).

The other special stains like Congo red, toluidine blue and Alcian blue were negative for toto bodies suggesting the absence of amyloid and mucins (Figs 3A to D).

CONCLUSION

Any diseased condition of the body causes many changes which vary according to its severity. Similarly, the inflammatory changes in the connective tissue influence changes in the epithelial cells that frequently result in the formation of eosinophilic bodies. Presence of these bodies is in favor of inflammatory reactions.

Various stains were used in order to study, demonstrate and describe the nature of the eosinophilic bodies. The stains which showed positivity for toto bodies were PAS, modified Papanicolaou, Masson's Trichrome and combined PAS–Pap technique which characterizes them mainly as glycoproteins and keratins. It was also observed that the Masson's trichrome staining demonstrated a better contrast of these bodies followed by combined PAS–Pap, PAS, and Pap. Therefore, it may be concluded that Toto bodies are best observed using Masson's trichrome stain and their differential staining depends on the nature and severity of inflammation present in a lesion.

Figs 2A to H: Photomicrographs of well-differentiated squamous cell carcinoma showing positively stained Toto bodies in various special stains; Masson's trichrome stain X100(A), X400(B) ; PAS-Pap X100(C), X400(D); periodic acid Schiff (PAS) X100(E), X400(F); Papanicolaou (PAP)X100(G), X400(H).

Figs 3A to D: Photomicrographs of pyogenic granuloma (A) H&E; (B) Alcian blue; (C) Congo red; (D) Toluidine blue showing negative stains for Toto bodies (X100)

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