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Influence of Inflammation to Lymphangiogenesis in Human Dental Pulp

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SUMMARY

During inflammation, lymphangiogenesis takes place to enhance the transport of filtered fluid, proteins, and immune cells. Dental pulp tissue is frequently exposed to inflammatory insults, but the lymphatic system of the pulp and its responses to injury have not been investigated in detail using specific lymphatic markers. The aim of this study was to evaluate and to compare the lymphatic system in health dental pulp and pulp with inflammation, and to establish whether lymphangiogenesis takes place during dental pulp inflammation.

Ten pulps with irreversibile pulpitis and eleven samples of healthy dental pulps were included in this study. All pulp samples were analyzed microscopically using the standard hematoxylin-eosin (HE) staining to detect the presence of inflammation. Immunohistochemical staining was performed using monoclonal anti-CD31 antibody (DAKO) at dilution 1:20. Microvessels identified by CD31, in which lumen the red blood cells were not detected, were considered as lymph vessels. Active areas of lymphangiogenesis ("hot spots") were selected using low magnification. Images from five high power fields in the hot spot areas were recorded for each sample. Lymph vessels were counted using ImageJ program. The total number of lymph vessels so obtained was then divided by the number of the counted hot spots, and the result was used to denote the lymph vessel density.

The mean number of lymphatic vessels, detected by CD31, in the group without inflammation was significantly lower than in the group with inflammation (3.75 versus 13.58, $t=7.093$, $p<0.001$).

The present study established an increased number of lymphatic vessels in the inflamed human dental pulp suggesting that inflammation contributes to lymphangiogenesis.

Key words: **pulp, inflammation, lymphangiogenesis**

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INTRODUCTION

The lymphatic system is important for immune barrier function and for tissue fluid balance (1). Lymphatic vessels are identified with specific lymphatic markers in the pulp, however, little has been known about their function so far. Because of the special circulatory conditions in the pulp, there are several clinical implications that need to be considered in dental treatment (1).

In human frozen sections, both enzyme-histochemical and immunohistochemical methods have demonstrated that large lymphatic vessels are located in the central part of the pulp, while small lymphatic vessels are found in the periphery of the pulp, suggesting that lymphatic drainage of the human dental pulp starts from the periphery of the pulp and collects in the central part of the pulp (2).

Recently, monoclonal anti-CD31 antibody and the vascular endothelial growth factor receptor-3 have been demonstrated to be the markers of lymphatic endothelium (3).

Considering that lymphatic vessels in the dental pulp play an important role in the drainage of excess tissue fluid, the purpose of the present study was to evaluate and investigate the lymphatic system in healthy dental pulp and pulp with inflammation, i.e. the presence of lymphangiogenesis in inflamed human dental pulp.

METHODOLOGY

Ten pulps with irreversible pulpitis and eleven samples of healthy dental pulps were included in this study. The healthy dental pulp extracted due to prosthetic reasons constituted the control group. All pulp samples were analyzed microscopically using the standard hematoxylin-eosin (HE) slides to detect the presence of inflammation. All specimens were immunohistochemicaly stained for CD31. Immunohistochemical method was performed to detect vascular spaces using monoclonal anti-CD31 antibody (DAKO) at dilution 1:20. Microvessels identified by CD31, in which lumen red blood cells were not detected, were considered as lymph vessels. Active areas of lymphangiogenesis ("hot spots") were selected using low magnification. Images from five high power fields in the hot spot areas were recorded for each sample. Lymph vessels were counted using ImageJ program. The total number of lymph vessels so obtained was then divided by the number of the counted hot spots, and the result was used to denote the lymph vessel density.

The t- test was used to estimate the expression of lymphatic vessels. The result was considered statistically significant if $p<0.05$. All analyses were performed with the SPSS statistical package (SPSS version 10.0 for Windows).

RESULTS

The present investigation included 21 samples of dental pulp. Ten patients with clinical manifestation of pulpitis and 11 dental pulp specimens, extracted due to prosthetic reasons, were treated and collected at the Clinic of Dentistry, Faculty of Medicine, Niš. The age of 21 investigated patients ranged from 12 to 60 years.

Immunostaining of the lymphatic endothelialium with monoclonal anti-CD31 antibody demonstrated lymphatic vessels in the human dental pulp. The mean number of lymphatic vessels positive for CD31 in the group with inflammation (13.58 ± 4.35) was significantly higher ($t=7.093$, $p<0.001$) than the mean number in the healthy dental pulp group (3.75 ± 0.42) (Table 1).

Table 1. *Lymphatics in healthy and inflamed dental pulp*

Parameters	Healthy dental pulp	Inflamed dental pulp	p
N*	11	10	
LV $\bar{x}\pm SD$**	3.75 ±0.42	13.58 ± 4.35	<0.001

N* - number of patients
LV** - lymphatic vessels

Figure 1 shows the presence of inflammatory cells in the dental pulp with pulpitis (Figure 1a, 1b) and lymphatic vassels, CD31 positive, in healthy dental pulps (Figure 1c) and human pulp with pulpitis (Figure 1d).

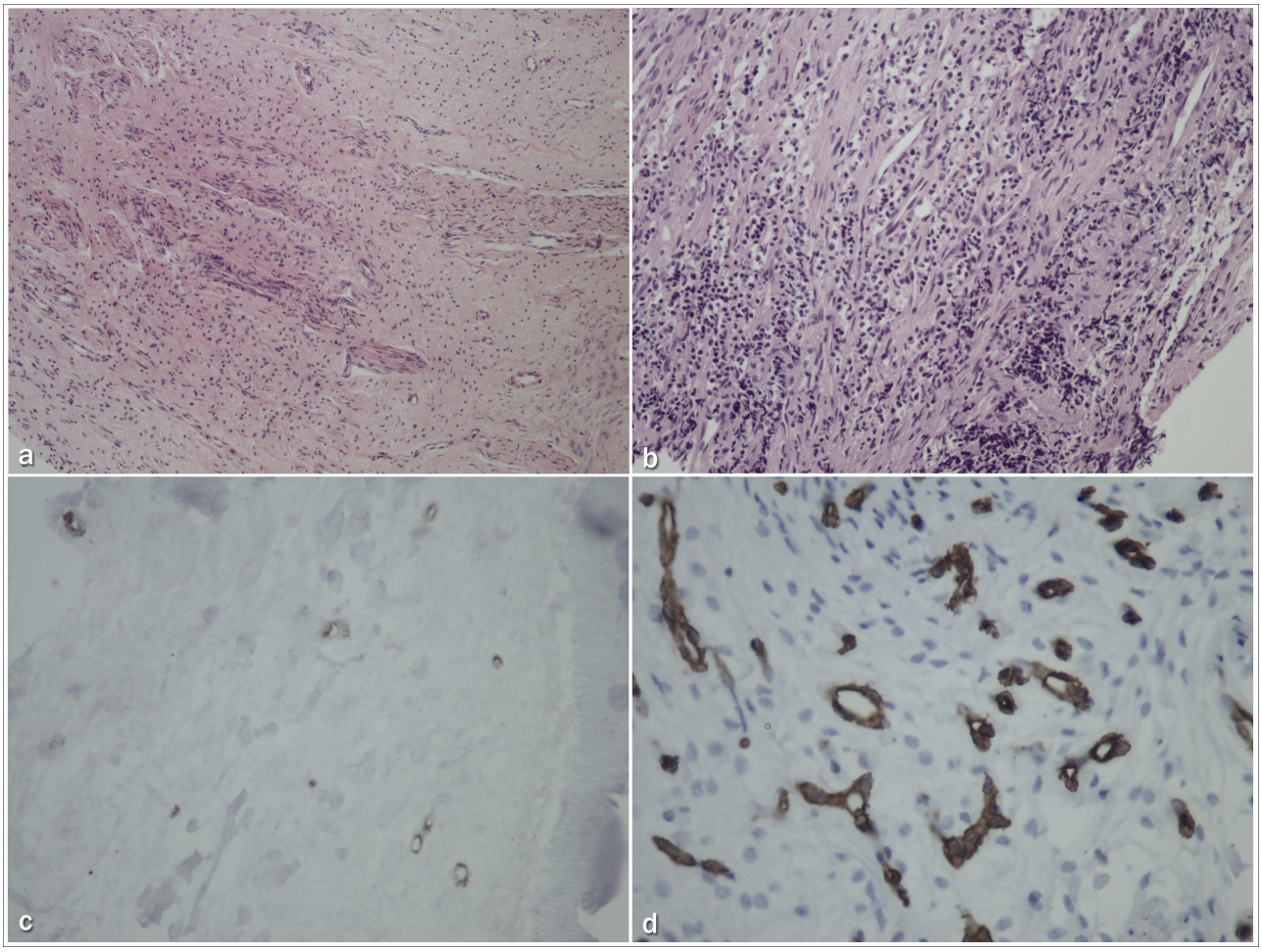

Figure 1. *Inflammatory cells in dental pulp with pulpitis detected with HE staining (1a, 1b) (HE, x200) and CD31 positive lymphatic vassels in healthy and inflamed dental pulps (1c, 1d) (En vision, x200)*

DISCUSSION

The dental pulp is a specialized loose connective tissue, containing cells, fibers, ground substance, blood vessels, and nerve endings. It is enclosed within rigid dentin walls and forms with the dentin an embryologic and functional entity known as dentinopulpar complex (4). The pulp tissue has several functions including initiation, formation, protection, nutrition, repair, and promotion of tooth vitality. Histologically, four distinct zones can be distinguished in the pulp tissue: the odontoblastic zone at the pulp periphery, a cell-free zone beneath the odontoblasts, a cell-rich zone in the area of pulpal tissue, and the pulp core where there are major vessels and nerves (4).

Although fibroblasts are the most predominant cell type in the dental pulp, other cell types can be observed as well, such as odontoblast, blood cells, Schwann cells, endothelial cells, and undifferentiated mesenchymal cells. Additionally, during inflammatory episodes, cells involved in the immune response, such as macrophages, mast cells, antigen-presenting cells, and plasma proteins may also be found (4).

Pulpitis is similar to inflammation in other tissues, however, it takes place within the rigid dentin walls. The acute inflammation is local, immediate reaction of the microcirculation, leading to increased blood flow, plasma exudation and escapement of white blood cells into the surrounding tissue. Chronic inflammation is characterized by cellular infiltrates, i.e. lymphocytes, plasma cells, macrophages and proliferation of capillaries and fibroblasts (5-7).

The dental pulp is more sensitive to changes in tissue pressure than other tissues, and it requires an active drainage system to eliminate excess fluid and macromolecular substances, and this effective system especially plays a great role in the inflammatory states (1, 3).

Various reports have demonstrated the presence of lymphatic vessels in the human dental pulp (1-3, 5-8). The existence of lymphatic vessels in dental pulp has been a matter of continuing controversy because of the difficulty of discriminating them in ordinary stained tissue sections (2). Recently, lymphatic vessels have been demonstrated by the presence of VEGFR-3 and CD31 in immunohistochemical reactions (3, 7, 10).

The present investigation has shown that human dental pulp, with clinical manifestation of pulpitis, has increased the number of lymphatic vessels compared to healthy dental pulp, with significant difference. This finding can be indicative for lymphangiogenesis in the human dental pulp during inflammation. The new lymphatic vessels could facilitate the removal of excess interstitial fuid, limiting the ejection of tissue pressure on connective tissue. However, further studies are needed to exclude the possibility that the part of lymphatic vessels in the group with inflammatory infiltrate were already present before inflammation, ie. it is not possible to determine the influece of lymphangiogenesis and dilatation of preexisting lymphatic vessels in drainage of excess interstitial fluid. The modulation of lymphatic vessel induction might be important for the human dental pulp regeneration and could be useful in conservative endodontic procedures, such as pulpotomy and direct pulp cupping.

The oral cavity is the site of many infectious and inflammatory diseases. Dental procedures (tooth extraction and endodontic treatment) can provoke the entrance of oral microorganisms of dental plaque into the blood flow and lymphatic system. This bacteriemia is short- term, but in the conditions of weakened defense mechanisms, the risk of these bacteria is increased. Some diseases, such as infective endocarditis, infections of head and neck, respiratory infections, diseases of gastrointestinal tract, skin diseases, bone diseases, occur as the consequence of the transmission of microbes from dental foci (11).

Prevention of dental pulp inflammation and consecutive deseases might be good restorative treatment of carious and noncarious defects of teeth. Marginal gap and alteration of enamel around adhesive restoration of teeth can provoke microleakage and entrance of oral fluid, bacteria, antigens to the pulp with inflammatory response of pulp tissue (12-14). Collateral blood vessels and lymphatic system must be of great importance in pathophysiology end prognosis of pulp infection. Well-developed collateral circulation may prevent the spread of inflammation and necrosis into dental pulp, as in other tissues (15). Because of the special circulatory conditions in the pulp, there are several clinical implications that need to be considered in dental treatment (16).

CONCLUSION

The present study established an increased number of lymphatic vessels in the inflamed human dental pulp suggesting that inflammation contributes to lymphangiogenesis.

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UTICAJ ZAPALJENJA NA LIMFANGIOGENEZU U ZUBNOJ PULPI LJUDI

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Sažetak

Tokom zapaljenja, limfangiogeneza doprinosi ubrzanom transportu filtrirane tečnosti, proteina i ćelija imuniteta. Tkivo zubne pulpe je često izloženo zapaljenjskom oštećenju, ali limfni sistem pulpe i njegovi odgovori na oštećenje nisu ispitani detaljno korišćenjem specifičnih markera za limfatike.

Cilj ove studije bio je da utvrdi i uporedi limfni sistem zdrave i pulpe sa zapaljenjem, kao i da utvrdi da li se limfangiogeneza javlja tokom zapaljenja pulpe.

Ovo ispitivanje je obuhvatilo deset pulpi sa ireverzibilnim pulpitisom i jedanaest uzoraka zdrave zubne pulpe. Svi uzorci pulpi su analizirani mikroskopski pomoću standardnog hematoxylin-eosin (HE) bojenja radi otkrivanja prisustva zapaljenja. Imunohistohemijsko bojenje je primenjeno za detekciju vaskularnih prostora, korišćenjem monoklonalnog anti CD31 antitela (DAKO) u razblaženju 1:20. Mikroskopski verifikovani vaskularni prostori, pomoću CD31 i u čijem lumenu nisu detektovani eritrociti, smatrani su limfnim sudovima. Aktivna polja limfangiogeneze ("hot spots") određena su pomoću malog mikroskopskog uvećanja. U svakom uzorku je zabeleženo po pet slika iz "hot spot" polja. Limfni sudovi su brojani pomoću ImageJ programa. Ukupan broj limfnih sudova, utvrđen na ovaj način, podeljen je brojem "hot spot" polja, a rezultat je predstavljao gustinu limfatika.

Prosečan broj limfnih sudova, detektovanih pomoću CD31, u grupi bez zapaljenja, bio je značajno manji nego u grupi sa zapaljenjem (3.75 u odnosu na 13.58, $t=7.093$, $p<0.001$).

Ovo ispitivanje je utvrdilo porast broja limfnih sudova u zubnoj pulpi sa zapaljenjem, što pokazuje da zapaljenje doprinosi limfangiogenezi.

Ključne reči: **pulpa, zapaljenje, limfangiogeneza**