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Do Parathyroid Glands from Individuals of Different Age and Gender Contain Lymph Vessels?

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A B S T R A C T

Whereas lymph vessels in some endocrine glands have been thoroughly investigated, data on these vessels in human parathyroid glands are often contradictory and deficient in available literature. Therefore, the aim of this study was to histomorphologically investigate whether lymph vessels could be found in human parathyroid glands postnatally and, if so, whether their presence was age- and gender-dependent. A total of 44 parathyroid glands from subjects of both genders, aged 4–90 years, were studied. The glands were divided into three groups. Those from the 1st and the 2nd age group demonstrated similar morphological structure of parenchyma with predominant chief cells with pale-staining cytoplasm, while the frequency of lymph vessels was lower in the 2nd group. Unlike in these groups, chief cells with dark- staining cytoplasm predominated in the glandular parenchyma of the 3rd age group where lymph vessels were not found in any of the examined glands. The frequency of lymph vessels in parathyroid glands was almost the same for both genders. Histomorphologic occurrence of lymph vessels coincided with the presence of endocrine cells with pale-staining cytoplasm, which allowed the assumption that lymph vessels were also one of the signs of functional activity of human parathyroid glands.

Key words: *human parathyroid glands, lymph vessels*

Introduction

Human parathyroid glands were discovered as late as at the end of the 19th century[1]. They are surrounded by a thin fibrous capsule. Their parenchyma consists of chief and oxyphil cells. According to the intensity of the cytoplasm staining and nuclear morphology in histologic sections and cytologic smears, chief and oxyphil cells occurr in 2 morphologic types, dark and light cells[2,3]. While ultrastructural and cytologic analyses of chief cells suggest their function, the function of oxyphil cells is still unknown[2–4]. Similar is with the presence of lymph vessels in the parathyroid gland.

Histomorphologic studies related to the origin, development, structure and function of lymph vessels are still lagging behind the studies of the same type that address the vascular system. This is probably due to differences in form, achromaticity of the lymph system, and to the histomorphologic structure of these vessels that allows their walls to be easily damaged and is thus a cause of additional problems.

Recent literature, however, provides sufficient amount of data on particular factors that affect the growth (vascular endothelial growth factor, VEGF) and permeability (vascular permeability factor, VPF) primarily of blood vessels but also of lymph vessels[5,6]. Both of these groups of factors belong to proteins. As there are several forms of endothelial growth factors, endothelial cells have specific receptors for each of them[7]. Thus, VEGFR-2 and VEGFR-3 receptors have been demonstrated on the lymph vessel endothelium, and they bind VEGF-C[8,9] and VEGF-D[6] endothelial growth factors. Apart from affecting the growth of lymph vessels in embryonic[7] and differentiated tissues, VEGF-C was reported to induce hyperplasia of the already existing lymph vessels involved in draining interstitial fluid during inflammatory reactions and tumor metastases[10].

VEGF-C and its receptor, VEGFR-3, were also shown to jointly affect the development of lymph vessels in adults[11], and to influence transient lymphangiogenesis that occurs concurrently with angiogenesis during wound healing[12].

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Excess of interstitial fluid is collected by the lymph and drained away in the blood. In addition, and hormones are excreted into, and transported by the lymph (lymphocrinia), which was confirmed by the findings of lymph vessels in some endocrine glands, e.g. in pancreatic islets of Langerhans, in adrenal glands, testicles, ovaries[13] and in the thyroid gland[14].

Unlike the endocrine glands above, lymph vessels of human parathyroid glands have been the topic of few histomorphologic studies that involved the period not only before birth but also later during life. As evident from the literature, even those few data that are available are often contradictory and deficient.

While lymph vessels in parathyroid glands were described by some authors as »wide lymph spaces in the vicinity of arteries«[15] or as internal or external capillary network in their capsule, some other authors stated that these vessels could rarely be observed[16] or their presence could not be found at all in the above-mentioned glands[17–19].

Due to all foregoing reasons, we initiated this study as a continuation of our previous investigation of lymph vessel development in human embryonic and fetal parathyroid glands[20] with the following aims:

– to investigate the existence of lymph vessels in human postnatal parathyroid glands;
– to verify, in the case of their confirmed existence, whether the presence of lymph vessels depends on age and gender.

Materials and Methods

Tissue samples for this study originated from the Department of Histology and Embryology, from the Department of Forensic Medicine and from the Department of Pathologic Anatomy, School of Medicine, University of Zagreb. The study included 44 parathyroid glands obtained 12–24 hours after death of individuals of both gender, aged 4–90 years (Table 1).

After being subjected to usual histologic preparations (fixation in 10% water formalin solution, rinsing, dehydration), glandular tissue samples were embedded in paraffin and serially cut into 5–7 μm thick sections. Serial cuts were stained with hemalaun and eosin, subjected to PAS method and light microscope study with ocular and objective magnification of x100 and x400, respectively. To verify the finding of lymph vessels in parathyroid glands, the sections with observed vessels were also studied using immersion, objective x100.

TABLE 1
DIVISION OF THE EXAMINED HUMAN PARATHYROID GLANDS ACCORDING TO THE AGE AND GENDER

Age groups	Number of examined glands		
	Males	Females	Total
I (4 – 30 years)	7	4	11
II (31–60 years)	9	5	14
III (61 – 90 years)	11	8	19

Results

Results of the study are presented for all three age groups in Table 2.

In the 1st age group, lymph vessels were detected in 10 of 11 examined glands. They were found in connective trabeculae between predominant chief parenchymal cells with the pale-staining cytoplasm, and outside the gland in the vicinity of the connective tissue capsule. Valves were observed in the lumen of some of these vessels (Figure 1a), while the lumen of others was partially filled with slightly eosinophilic content.

Lymph vessels in the 2nd age group were found in 11 of 14 examined glands, and were situated in or outside the gland beside the outer surface of the capsule. Some of them were detected close to blood vessels (Figure 1b), others were in connective trabeculae or in the parenchyma between predominantly chief cells with the pale- staining cytoplasm (Figure 1c). Similarly to the 1st age group, some of these vessels had, along with valves, the lumen filled with slightly eosinophilic content. Lymph vessels were in both investigated groups of different forms regardless of their location. Thus, e.g., some were lacuna-shaped, some elongated with narrow lumen (Figure 1b).

Lymph vessels were not detected in any of the examined glands from the 3rd age group. Indeed, there were structures that resembled lymph vessels by their histomorphological architecture. However, the examination and immersion verification demonstrated that the observed structures were individual lipid cells or vessel artefacts. Chief cells with dark-staining cytoplasm were predominant in the glandular parenchyma.

Results of the frequency of lymph vessels in parathyroid glands regarding the age are presented in Table 2. Lymph vessels were found in 48.15% of 27 examined glands from male individuals and in 47.06% of 17 glands from female individuals.

TABLE 2
THE FREQUENCE OF LYMPH VESSELS IN HUMAN PARATHYROID GLANDS ACCORDING TO THE AGE AND GENDER

	Age			Sex	
	I (4–30)	II (31–60)	III (61–90)	Males	Females
Number of examined parathyroid glands	11	14	19	27	17
Number of parathyroid glands with lymph vessels (%)	10 (90.90%)	11 (78.60%)	0 (0%)	13 (48.15%)	8 (47.06%)

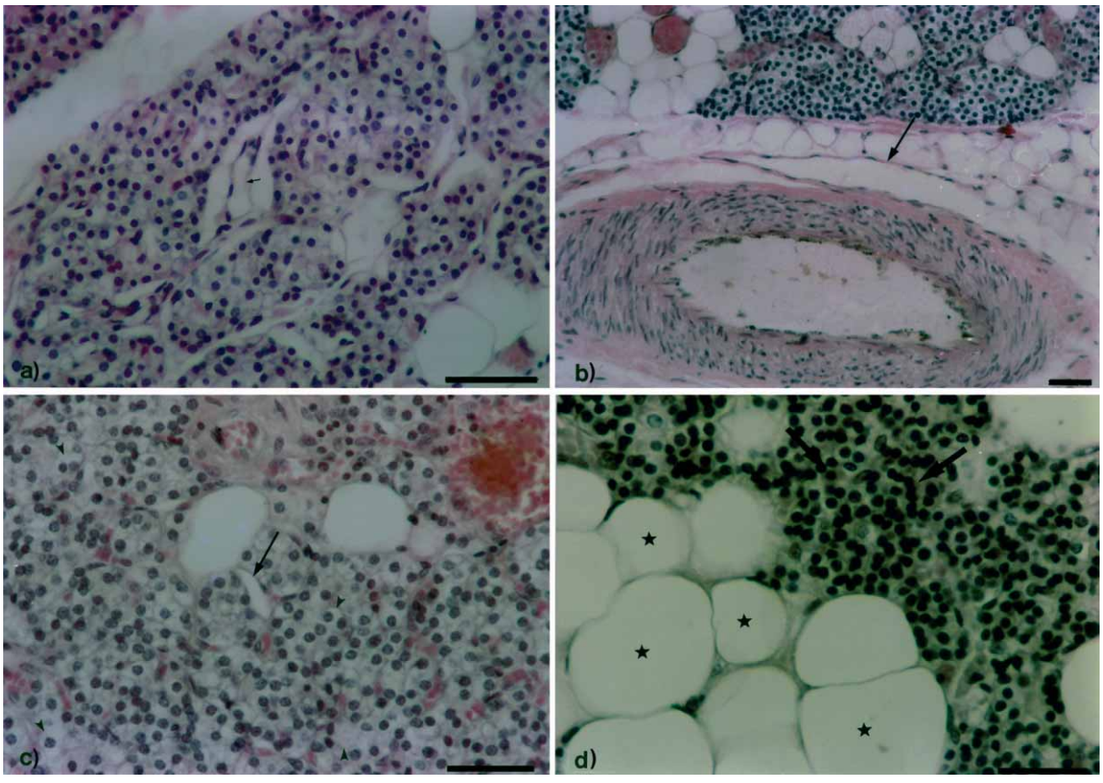

Fig. 1. a) Lymph vessel with a valve (small arrow) in the parathyroid gland of a male age 26. H. & E., x200, scale bar = 100 μm, b) Blood and lymph vessel (arrow) attached to the connective capsule in the parathyroid gland of a male age 48. H. & E., x100, scale bar = 100 μm, c) Lymph vessel (arrow) surrounded by chief cells with pale-staining cytoplasm (arrowhead) in the gland of a male age 59. H. & E., x200, scale bar = 100 μm, d) Clusters of fat (star) and chief cells with dark-staining cytoplasm (arrow) in the parathyroid gland of a woman age 77. H. & E., x200, scale bar = 100 μm.

Discussion

Parathyroid glands have been recently studied mainly from the clinical aspect associated with different endocrine and bone system diseases. However, there has been insufficient interest in some histomorphological structures in these glands as, e.g., the presence of lymph vessels. There were scarce histomorphologic descriptions of lymph vessels in human parathyroid glands in the past and in the recent available literature. Nevertheless, these descriptive studies referred mostly to their form, location and distribution, whereas either age or gender of individuals in whose glands lymph vessels were detected were not reported[1,13,15,16].

Similar situation was observed with descriptions of lymph vessels in some other endocrine glands, and the assumption of authors of previous studies was that both blood and lymph vessels served in those glands for the hormone transport[13]. This assumption was recently confirmed based on a series of studies on animals[21-23] and humans[24,25]. However, we could not find reports on studies associated in this respect to lymph vessels in human parathyroid glands in the available literature.

This study included 44 parathyroid glands from individuals of different age and sex. All investigated material was divided according to three age groups and in two groups according to gender (Table 1).

Histomorphologic structure of parathyroid glands in the 1st and 2nd age group shared a number of properties:

- parenchyma of examined glands consisted mostly of chief cells with pale–staining cytoplasm;
- lymph vessels were found both in and outside the glands close to the connective tissue capsule;
- the form and the content of lymph vessels were similar in both groups independently of their location;
- valves were observed in some of the lymph vessels mentioned above.

These histomorphological findings were consistent with results of few authors who described lymph vessels in parathyroid glands[13,15,20].

Comparison of the proportion of lymph vessels between the 1st and the 2nd age group demonstrated lower proportion of these vessels in the 2nd age group. Actually, lymph vessels were present in 90.9% and 78.6% of glands from the 1st and the 2nd age group, respectively. Unex-

pected result was the fact that none of lymph vessels were found in the glands of the 3rd age group despite the highest number of samples examined (Table 2).

Since no data were found in the literature with which to compare our findings, no specific statement can be made about either the cause of the diminished proportion of lymph vessels in glands from the 2nd age group or their absence in the 3rd age group. Only some assumptions could be outlined.

If hypotheses of some authors that hormones may be transported by blood- and lymph vessels are considered[13], then the deficiency of lymph vessels in examined parathyroid glands of individuals from the 3rd age group might point to changes in relations that occur between bone tissue on the one hand and parathyroid glands on the other at the respective age.

The hormone of parathyroid glands is stored in secretory granules within active chief cells with pale-staining cytoplasm. PTH, D vitamin and calcitonin are basic regulators of calcium and phosphorus turnover which is important for the maintenance of normal structure of bone matrix during lifetime. The bone metabolism is also affected by estrogens[26] and androgen hormones[27]. Due to the reduced level of these steroids, excessive bone degradation or reduced bone production (postmenopausal and senile osteoporosis) occur at an advanced age. Since each elevation of plasma calcium concentration lead to reduced PTH excretion, reduced excretion of PTH could be expected at an advanced age due to enhanced bone degradation and calcium release. This conclusion is supported by some histomorphologic findings obtained in this study from individuals from the 3rd age group. In the literature, morphologic types of chief cells are described for the light microscopic (histologic sections and cytologic smears)[3] and electron microscopic preparations of normal human parathyroid glands[2,4]. Dark chief cells have slightly eosinophilic, scarce cytoplasm and centrally located nucleus with condensed chromatin. Ultrastructurally they have glycogen granules, a small Golgi complex, a few secretory granules and less prominent endoplasmic reticulum[2,4]. These characteristics suggest the resting phase of chief cells. Light chief cells contain more abundant cytoplasm and a larger nucleus, with looser chromatin and 1 or 2 visible nucleoli[3]. Ultrastructurally numerous small granules, prominent Golgi complex and parallel cisternae of the rough endoplasmic reticulum suggest the active phase of chief cells[2,4]. Actually, it has been widely recognized that chief cells with pale-staining cytoplasm and larger nuclei are active, i.e. they excrete PTH, while chief cells with dark-staining cytoplasm and small nuclei are dormant. As the glandular parenchyma of the 3rd age group was composed mainly of dark cells, it appears that PTH excretion could also be reduced. If the fact that hormones could be transported by lymph is accepted, reduction in those structures that are used for the hormone transport could be expected in the case of a hormone level reduction. In this histomorphologic study, such structures were lymph vessels.

As the examined parathyroid glands in the 1st and 2nd age group were made up of mostly chief cells with pale-staining cytoplasm, which indicated glandular activity, the finding of lymph vessels in these glands was in accordance with the previously stated assumptions. Actually, this could indicate that the need for PTH transport was higher at these age groups than at advanced age due to the rapid bone turnover and maintenance of the bone matrix. However, secondary hyperparathyroidism often occurs at an advanced age as a result of reduced calcium resorption in intestinal epithelium and distal renal tubules. This fact should warrant the expectation of an increased number of chief cells with pale-staining cytoplasm in parathyroid glands of elderly persons, which is in disagreement with our histomorphologic findings.

The issue that still remains indefinite is the fact that none of the available reports stated the point during lifetime until which the lymphoangiogenic growth factor VEGF-C generally affects the development of lymph vessels, and thus also of those from parathyroid glands. Some literature data stated that biological activity of VEGF-C depended on angiogenic regulators present in the microenvironment of endothelial cells to which they respond[28]. There are also opinions that angiogenesis inhibitors, i.e. »negative vessel growth regulators« (the author did not emphasize that he referred to blood vessels only), are members of a large group of suppressors that prevent the mitosis of endothelial cells[29]. According to the same author, endothelial proliferation may be prevented by pericyte activity, mitogen removal from extracellular matrix, altered endothelial forms that reduce its sensitivity to growth factors, or by the activity of certain endothelial integrins.

During their study of VEGF-C, Cao et al.[30] observed that it could provoke changes in endothelial forms and actin reorganization in endothelial cells containing VEGFR-2 and VRGFR-3 receptors, but not in those containing VEGFR-1 receptor. According to their opinion, VEGFR-2 and VEGFR-3 could change proliferative and chemotactic responses of endothelial cells to stimulatory activity of VRGF-C. However, unlike VRGFR-1 and VEGFR-2, VEGRF-3 was expressed mostly in endothelial cells of lymph vessels from tissues of adults, and very poorly in other vessels. The authors believed that angiogenic activity of the growth factor *in vivo* depend on the balance between angiogenic factors and inhibitors in the tissue.

Based on the data from available literature, the possibility that some of the stated factors could also affect the reduction of lymph vessels in parathyroid glands of the 2nd age group, or their absence in the 3rd age group, could not be ruled out.

Results of investigating the frequency of lymph vessels in parathyroid glands with regard to gender demonstrated that the finding of these structures was almost equal for both genders (Table 2). The slight difference observed might only be fictitious. These results should be accepted critically on account of the uneven number of glands from individuals of both genders; even more so

because available literature provided no similar studies for the comparison with our results.

Histomorphologic occurrence of lymph vessels coincided with the presence of endocrine cells with pale-staining cytoplasm, which allowed the assumption that lymph vessels were also one of the signs of the functional activity of human parathyroid glands.

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IMA LI LIMFNIH ŽILA U EPITELNIM TJELEŠCIMA LJUDI RAZLIČITE ŽIVOTNE DOBI I SPOLA?

SAŽETAK

Dok su limfne žile u nekim endokrinim žlijezdama dobro istražene, podaci u dostupnoj literaturi o spomenutim strukturama ljudskih epitelnih tjelešaca često su oprečni i manjkavi. Stoga je cilj ovoga rada bio histomorfološki istražiti postoje li limfne žile u epitelnim tjelešcima čovjeka nakon rođenja i ako postoje, ovisi li njihova prisutnost o životnoj dobi i o spolu. Istraženo je 44 epitelnih tjelešaca osoba oba spola, od 4. do 90. godine života. Žlijezde su podijeljene u tri dobne skupine. Prva i druga dobna skupina pokazuju sličnu morfološku građu parenhima u kojem su prevladavale svijetle glavne stanice, dok je učestalost limfnih žila manja u drugoj skupini. Za razliku od prethodnih skupina u žljezdanom parenhimu treće dobne skupine prevladavale su tamne glavne stanice, a limfne žile nisu pronađene ni u jednoj istraženoj žlijezdi. Učestalost limfnih žila epitelnih tjelešaca gotovo je jednaka u oba spola. Histomorfološka pojava limfnih žila podudara se s prisutnošću svijetlih endokrinih stanica, što dopušta pretpostaviti da su i limfne žile jedan od znakova funkcionalne aktivnosti epitelnih tjelešaca čovjeka.