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The Development of Blood and Lymph Vessels of Human Parathyroid Glands in Embryonal, Fetal and Postnatal Period

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A B S T R A C T

The aim of the article is to investigate the development of blood and lymph systems in human parathyroid glands in prenatal and postnatal periods. The first capillaries are observed in these glands already in the lunar month 2. At the middle of pregnancy blood supply is increased, being extremely abundant in lunar months 9 and 10, as well as during the first year of life. As parts of the lymph system, intercellular lymph spaces are noticed in the parathyroid glands already in the lunar month 2, and also later, when lymph vessels are situated along the gland or in its connective capsule and within the gland parenchyma respectively. All these findings could be connected with the early function of these glands, as well as with the possibility that parathyroid hormone (PTH) is not transferred by blood only but by lymph as well.

Introduction

Although parathyroid glands were discovered in the Indian rhinoceros (Rh. unicornis)[1] in 1850, and in man[2] in 1880, still a lot has remained unclear about their development and structure. Open are the questions at what time blood vessels in parathyroid glands of human embryo and fetus could be traced at the earliest, and specifically whether in these glands lymph vessels do exist at all and,

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if they do, when they can be seen. Therefore, the aim of this work is to investigate the development of the mentioned structures in human parathyroid glands in pre- and postnatal periods.

There are various data in older literature about the time of emerging of *blood vessels* during the development of these glands. Keibel and Mall[3] state that they observed the first blood capillaries in the tender connective tissue of a human embryo 9.75 mm long (lunar month 2).

Norris[4] engaged himself with the development and histological differentiation of blood vessels in human parathyroid glands. The author states that in the embryo 15 mm long (l.m. 2) several blood capillaries among gland cells could be noticed. According to his opinion, their blood supply increases with their development, although in an embryo of 20–100 mm length (l.m. 2–4) it is still rather scanty. Only in an embryo 100–150 mm long (l.m. 5) he noticed the development of numerous blood capillaries. According to Bargman[5] parathyroid glands of older fetuses are permeated with a net of capillaries.

In the sixties of the 20th century the data on time of occurrence of blood vessels in the respective glands in men are still discordant. Nakagami et al[6] cite the phenomenon of a tender connective tissue abundantly permeated with blood capillaries in parathyroid glands of human fetuses of 500 and 730 g weight (l.m. 6 and 7). Contrary to that, Valdis-Dapena[7] observed growing in of the capillary net in the bases of these glands in the 6th week of human embryo development (l.m. 2). Gouget et al[8] think that the capillaries are developed in the mentioned glands already at the end of the embryonal period.

Except Bargman[5], none of the cited authors mention the data on the development of *lymph vessels* in the parathyroid glands of human embryos and fetuses. Even Bargman[5] does not give his own observations, but quotes the findings of older authors like Schreiber[9] and Petersen[10].

Schreiber[9] noticed »wide lymph spaces« in the vicinity of arteries, while Petersen[10] stated that in the human parathyroid glands lymph vessels could be rarely found. Bargman[5] does not say whether this applies to the glands of adults, children or embryos and fetuses respectively.

Later on, Balshev and Ignashkina[11], studying lymph vessels of 10 human thyroid glands and parathyroid glands in adults, found outer and inner net of lymph capillaries in the capsule of parathyroid glands. Some of the inner net capillaries permeated into gland parenchyma together with connective tissue and blood vessels. Lymph capillaries of the outer net continued into efferent lymph vessels, or tied themselves with thyroid lymph vessels through anastomoses. However, these authors do not cite whether this is the case of glands in adults or of glands from pre- or perinatal period.

Although in the last decades a great advancement has been achieved in investigating the lymph system of endocrine glands, the data on this system in parathyroid glands in man are, as the literature shows, greatly insufficient, especially for embryonal and fetal periods.

These incomplete and controversial data that we have found in the available literature urged us to investigate the collected sample of embryonal, fetal, neonatal and infant parathyroid glands in order to contribute in elucidating the questions:

1. when do blood vessels appear within parathyroid glands during their histological differentiation;
2. do lymph vessels exist in parathyroid glands of human embryos, fetuses and

children in the first year of life, and if they do, when they can be observed at the earliest.

Material and Methods

The investigation has been performed on 17 embryonal and fetal parathyroid glands from the 2nd to the 10th lunar months (l.m.), and on 5 glands in the first year of life.

In order to obtain the accurate data on the embryo and fetus, where it was possible, we measured the embryo's crown-rump length (CRL) and the fetus' crown-heel length (CHL). Where measuring was not possible, the age of the embryo and fetus was determined by the size of the uterus and by the date on the last menstruation.

The tissue samples were fixated in 4% and 10% formalin and Bouin, inserted into paraffin and serially cut in 5–7 m thick slices. Serial sections were stained by HE, PAS method and Azane after Heidenheim, and investigated by light microscope and magnifying glass.

The tissue samples were obtained from embryos and fetuses after spontaneous abortions, premature births or from children in the first year of life who died due to heart failures.*

Results

Prenatal period

The first blood capillaries bounded by endothelial cells and filled with a few erythrocytes are observed in human parathyroid glands already in the 7th week of the embryonal development, i.e. in the lunar month 2. The blood supply of these glands is particularly increased during the next lunar months, and is specially expressed in the lunar months 9 and 10 (Table 1).

In lunar months 2 and 3, the blood capillaries are situated among gland parenchyma cells. In the lunar month 4, the extremely profound blood supply is noticed, particularly at the marginal parts of the glands, i.e. at the border towards connective capsule (Figure 1B). From 5th to 10th l.m. blood vessels (arteries and veins) are within well developed particles of the connective tissues, situated among gland cells.

Regarding the investigation of the lymph system in the human parathyroid glands, intercellular lymph spaces are observed already in the lunar month 2, sharply divided by tender connective tissue from the gland parenchyma (Table 1). The same spaces are present in the glands in the 3rd (Figure 1A), 6th, 8th and 9th lunar months. In these spaces the lumen is partly filled by a light rosy content.

In the immediate vicinity of the connective capsule of the investigated parathyroid glands of fetuses 3 to 7 lunar months old, well developed lymph vessels are observed, with a pronounced endothelium and filled with the light rosy lymph content.

A similar structure with lumen covered by endothelium, only without content, is visible in the parathyroid gland capsule in the 5th lunar month (Figure 1C).

In the parathyroid glands of the fetuses in lunar months 8 and 9 are ob-

* In obtaining and processing this material a lot of effort has been invested through years. This is the archive material of the Department of Histology and Embryology, School of Medicine, Zagreb, Croatia and is the collection of histological serial sections, thus being a rare and, due to that, extremely valuable object of interest for embryological, histomorphological, histometric, histochemical research. The results of a large number of studies performed on this material have been published in Croatia and abroad.

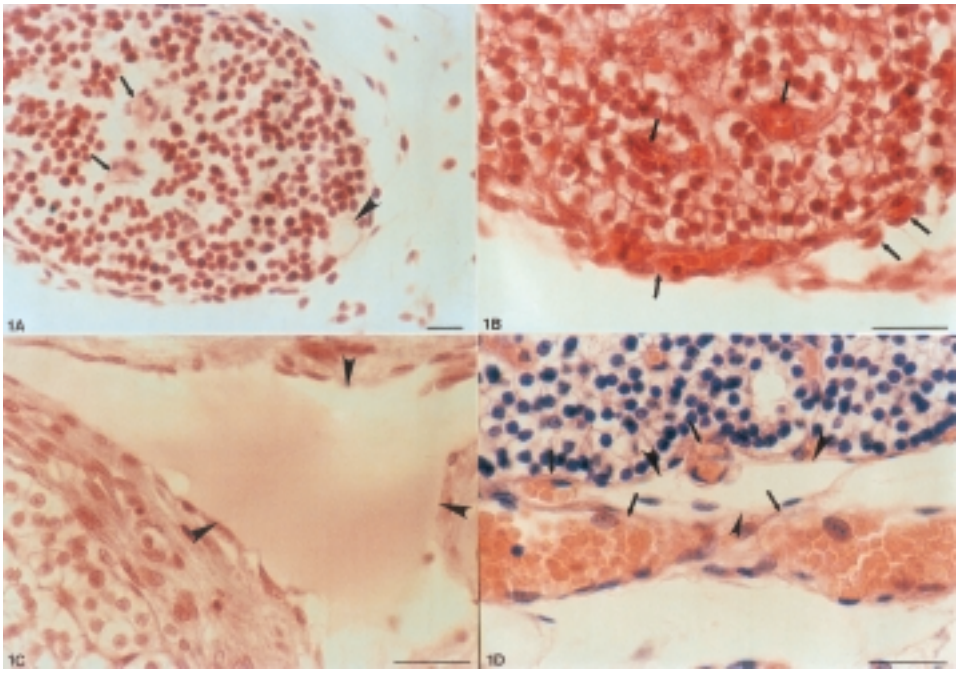

Fig.1. Human parathyroid gland in prenatal and postnatal period.

A. Parathyroid gland in the 3rd l.m. An intercellular lymph space (arrowhead) could be seen at the periphery of the gland. Blood vessels (arrows). H. & E., 100, bar = 100 m.

B. Part of the parathyroid gland in the 4th l.m. Numerous small blood vessels (arrows) within the glandular tissue and the capsule are visible. H. & E., 400, bar = 50 m.

C. Lymph vessel in the human parathyroid gland (the 5th l.m.) (arrowheads). Lymph vessel is lined with flattened endothelial cells. H. & E., 400, bar = 50 m.

D. Parathyroid gland in a newborn, detail. Lymph (arrowheads) and blood vessels (arrows) could be clearly distinguished. H. & E., 400, bar = 50 m.

served (along with already described intercellular lymph spaces without endothelium) structures within glands covered by endothelium, which by material and content correspond to lymph vessels.

Postnatal period

The postnatal period comprises 2 glands in neonatal, and 3 in infant age. Results leading to blood supply of parathyroid glands of children in the first year of age, regardless whether these are newborns or infants, are similar to the results of fetal glands in lunar months 9 and 10. All glands are extremely well supplied by blood, both in peripheral and in median parts. Arteries penetrate into glands within the connective tissue septa, abundantly ramify in thin branches up to blood capillaries, situated among gland cells (Figure 1D). In connective tissue septa are also veins that lead the blood away from the gland.

In 4 out of 5 parathyroid glands from the postnatal period are observed structures that by their material correspond to lymph vessels. Those are glands in 2 newborns and in 2 infants.

TABLE 1
SURVEY OF THE DEVELOPMENT OF BLOOD AND LYMPH VESSELS IN HUMAN PARATHYROID GLANDS IN PRENATAL AND POSTNATAL PERIOD

Age	Number of embryos and fetuses	Blood supply	Lymph system			
			Intercellular lymph spaces	Lymph vessels		
				Around the gland	In the capsule	Within the gland
l.m. 2	2	+	+	–	–	–
l.m. 3	2	+	+	+	–	–
l.m. 4	2	+	–	++	–	–
l.m. 5	2	+	–	++	+	–
l.m. 6	2	++	+	+	–	–
l.m. 7	2	++	–	+	–	–
l.m. 8	2	++	+	–	–	+
l.m. 9	2	+++	+	–	–	–
l.m. 10	1	+++	–	–	–	–
New-borns	2	+++	–	–	+	–
Infants	3	+++	–	+	+	–

l.m. = lunar month

The mentioned lymph vessels are found in the inner layer of the connective tissue capsule at the border towards gland parenchyma in the glands of both newborns. In their vicinity, both towards parenchyma and surrounding connective tissue, are smaller and bigger blood vessels (Figure 1D).

In the parathyroid glands of both infants, lymph vessels are also observed. In one infant they are situated in the inner layer of the connective tissue capsule surrounding the gland. In the other infant the same structure is noticed in the connective tissue septa within the gland itself, separated by a thin wall from the blood vessel.

Discussion

Terms vasculogenesis, angiogenesis and lymphangiogenesis are often met in the literature. Vasculogenesis designates the manifestation of first blood vessels from the mesenchyma, and angiogenesis the appearance of new blood vessels from the already existing ones. In human embryo during the 17th day after fertilization starts extraembryonal, and on the 18th day embryonal vasculogenesis.

Lymphangiogenesis denotes the emergence of lymph vessels. The results of early investigations of the origin and development of lymph vessels in man are contradictory. Thus, some authors considered that lymph vessels rose by budding from veins[12,13], that they grew in centrifugal direction and mutually bound, rapidly spreading into surroundings. Others, however, believed that lymph vessels originated in the mesenchyma from the spaces connecting in centripetal direction and only later opening up in veins[14,15]. There were opinions that lymph vessels were developing both from veins and by connecting the spaces in the mesenchyma.

According to recent opinions[17], in man first occur 6 primary lymph bags, mutually connected by lymph vessels. Lymph vessels run along large veins, with which

they finally unite[18]. However, it is still unclear whether lymph vessels occur by budding from venous endothelium, from lymphangioblasts, or in both ways.

Today is accepted the opinion that lymph vessels appear during the 5th week of embryonal development, although according to some authors[20] they occur somewhat later, i.e. from the 6th to the 7th week of embryonal development.

By investigating vasculogenesis and angiogenesis, special factors that effect the growth (VEGF – vascular endothelial growth factor) and permeability of blood vessels (VPF – vascular permeability factor) have been discovered[21,22]. Both groups of factors are proteins coded by the same VEGF genome. There are several forms of vascular endothelial growth factor. Up to date are known 5 types of human VEGF mRNA that code VEGF forms. For the mentioned factors there are special receptors resembling VEGF thyrosine-kinase, e.g. VEGFR-1, VEGFR-2 and VEGFR-3. The presence of VEGFR-1 and VEGFR-2 receptors is most strongly expressed in the endothelial cell membrane of the blood vessels. It is interesting that the same receptors are met in other cell types, e.g. in trophoblast, mesangial kidney cells, megakaryocytes and hematopoietic stem cells[22]. According to some authors, the VEGF receptors are indispensable for embryonal development of vessels[21,23], and for physiological and pathological angiogenesis[23].

Especially interesting are results of Kevin et al[21], who observed that during the fetal development in mice the receptor expression significantly decreases. In their opinion that could mean that in mice some other factor, and not VEGF, influences the blood vessels growth during fetal development, or some other VEGF receptor is expressed.

In the lymph vessels endothelium are proven VEGFR-2 and VEGFR-3 receptors. Endothelial vascular growth factors VEGF-C[19] and VRGF-D[22] are bound to them. As the investigations have shown that VEGF-C influences the lymph vessels growth[23,24], it is considered as the lymphangiogenic growth factor[25], both in embryonal and in already differentiated tissues[19,26].

The investigations of mouse embryos of various developmental ages have proved a strong VEGFR-3 expression in the vessel net in the neck, in the mesenterium and in the mesenchyma around metanephros[27]. All those are sites where first lymph vessels appear[28]. As seen from the available literature, vasculogenesis and lymphangiogenesis generally start in a human embryo already in the first and second lunar month respectively. However, in the literature are still insufficient data on the onset and nature of these processes in parathyroid glands and in some other endocrine glands in human embryos and fetuses.

According to our results, the first blood capillaries are noticed in the parathyroid glands of the human embryo already in the lunar month 2, what is in accordance with the findings of some other authors[3,4,7,8]. If taken into account that the parathyroid hormone (PTH) does not go through the placenta from the mother's blood into the fetal blood[29], it may be expected that the fetal parathyroid glands are producing it. This has been pointed out by some other studies, the results of which have spoken for the hypothesis that these glands are already active in the 2nd or 3rd lunar month[30,31].

Further more, from our results it is visible that the blood supply of these glands is increasing somewhere in the middle of pregnancy, being specifically good in lunar months 9 and 10, as well as during the first year of life. These findings agree with our preliminary results in human fetal testicles where the in-

crease in length of blood vessels was observed from the 16th to the 34th week of fetal life.

Increased blood supply of parathyroid glands don't need to be connected only with the increased function of gland cells, but also with an invasion of connective tissue in which blood vessels enter the gland[30]. However, the possibility that other angiogenic factors or receptors for VEGF in fetal period have a greater effect on the growth of blood vessels (as pointed out by Kevin et al.[21]) cannot be excluded.

Regarding the investigation of the lymph system in parathyroid glands in prenatal period, we have found intercellular lymph spaces already in the 2nd, and later in the 3rd, 6th, 8th and 9th lunar months. These intercellular lymph spaces, divided by tender connective tissue from gland cells of parathyroid glands would correspond to Semeina's description of lymph capillaries in human thyroid gland[32]. According to this author's findings, lymph capillaries in thyroid glands of human fetuses and children in the first and second year of life have the shape of a »lacuna« or »wide sinuses«. Later and at older age their number and outline change[32]. The appearance of described intercellular lymph spaces in parathyroid glands during lunar month 2 in our research would be in accordance with the data on early development of lymph system in other parts of the human embryo[17,20].

Our findings of lymph vessels, both in fetal and in postnatal periods, whether within the parenchyma, capsule or within immediate vicinity of these glands, could be connected with the gland cells function. If considered that these glands are active with changing intensity already from the 2nd lunar month[30] and throughout the whole fetal period and onwards, it would be expected that there are, besides blood vessels, developing lymph vessels as well. Moreover, many authors think that the arrangement of the lymph capillaries net depends on the structure and function of particular organs[11,32,33]. All these findings, including ours, are accordant with citations of Balashev and Ignashkina[11], who believe that there is a possibility that PTH, as well as hormones of some other endocrine glands, is not transferred only by blood, but by lymph as well.

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RAZVOJ KRVNIH I LIMFNIH ŽILA EPITELNIH TJELEŠACA ČOVJEKA U EMBRIONALNO, FETALNO I POSTNATALNO DOBA

S A Ž E T A K

Ovim se radom željelo istražiti razvoj krvnog i limfnog sustava u ljudskim epitelnim tjelešcima u prenatalno i postnatalno doba. Prve krvne kapilare zamijećene su u ovim žlijezdama već u drugom lunarnom mjesecu. Sredinom trudnoće povećavala se je prokrvljenost ovih žlijezda, a izuzetno je dobra u devetom i desetom lunarnom mjesecu, kao i u prvoj godini života. Od limfnog sustava zamijećeni su u epitelnim tjelešcima već u drugom lunarnom mjesecu, a i kasnije, intercelularni limfni prostori, zatim limfne žile smještene uz žlijezdu ili u njezinoj vezivnoj čahuri, odnosno unutar žljezdanog parenhima. Svi ovi nalazi mogli bi se dovesti u vezu s ranom funkcijom ovih žlijezda, kao i s mogućnošću da se PTH ne prenosi samo krvlju, već i limfom.