Histomorphometric Study of Axillary Lymph Nodes in Different Age Groups

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Abstract

Background Although some age-related changes in lymph node histoarchitecture have been described, they are seldom taken into account in traditional depictions of lymph nodes. Recently introduced clinical procedures, such as intranodal vaccinations have demonstrated the need for an accurate knowledge of the degenerative processes of lymph nodes. It is thus deemed necessary to obtain a detailed insight into anatomical changes within the lymph node throughout life as age-related degeneration can have a strong impact on the outcome of these new therapeutic strategies.

Aim To study the size and shape of the lymph nodes and to establish the age-dependent histoarchitectural changes in the lymph nodes in different age groups.

Materials and Methods A cross-sectional study was conducted in a total of 35 axillary lymph nodes. The adult axillary group of lymph nodes were from subjects aged between 18 and 70 years. The fetal lymph nodes were collected from 8 stillborn fetuses between 37 and 42 weeks. Thickness of the cortex and diameter of the germinal centers were measured using ocular and stage micrometer.

Results None of the fetal lymphocytic follicles showed evidence of a prominent germinal center. The germinal centers of young adults were not only more numerous but also larger in size when compared with the old. An age-related involution of the paracortical region was witnessed in the axillary lymph nodes. No evidence of lipomatous atrophy was encountered in any of the fetal lymph nodes. Interesting evidence of it was encountered in younger age groups. However, this was the most prominent feature in the older groups.

Keywords

► germinal center
► lipomatous atrophy
► paracortical region

Introduction and Background

The lymphatic system constitutes the major part of the peripheral lymphoid tissues. Lymph nodes are anatomically and functionally composed of three distinct areas: the cortex, paracortex and medulla. The cortex is a B cell—dependent area containing primary and secondary follicles. Primary follicles are aggregates of small lymphocytes. Secondary follicles are formed by a “pale” germinal center encircled by a dark, thick border of small B-lymphocytes. The paracortex surrounds the follicles and is interposed between the cortex and the medulla; it is a T cell—dependent area, formed by small lymphocytes. The medulla has sinuses from where the lymph is conveyed toward efferent lymphatic ducts and is a B cell—dependent area.

Certain normal immune functions decline with age. It is clinically important that with a decrease in immunologic vigor, the incidence of infections, autoimmune and immune complex diseases, and cancer increases (Mackay1 and Good et al2). Also, lymph node degenerative features

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as a potential predictor of oncological prognosis have been indicated by many studies. What remains an enigma is whether the diseases compromise normal immune functions, or whether a decline in normal immune functions to threshold levels predisposes individuals to diseases. Although some age-related changes in lymph node histology have been described, they are seldom taken into account in traditional depictions of lymph nodes. This is clinically important because of the crucial role of these organs in the immune system.

Classical desensitization in allergic patients takes several years and demands a large number of injections causing compliance problems. Recently introduced clinical procedures, such as intranodal vaccination in humans acts as a very effective substitute for the common method of allergen desensitization by reaching similar results in a much shorter time period with a total of only three intranodal injections (Senti et al.). Intranodal vaccinations have elicited potent prophylactic and therapeutic antitumoral immunity, resulting in a remarkable survival benefit. Therefore, it is of prime importance that the lymph nodes be evaluated for degeneration before such interventions. Hence, this study was structured to compare the histological features of lymph nodes in the different age groups to the study and evaluate the degenerative process of lymph nodes. These recently introduced clinical procedures have demonstrated the need for an in-depth knowledge of the degenerative processes of lymph nodes. It seems important to obtain a detailed insight into distinct anatomical changes within the human lymph node throughout life as degeneration can have a strong impact on the outcome of these new therapeutic strategies.

**Aim**

To establish the age-dependent histological changes in the lymph nodes.

**Objectives**

To compare the salient histological features of lymph nodes like germinal centers, paracortical region, and lipomatous atrophy in three different age groups.

**Materials and Methods**

A cross-sectional study was conducted with a total of 35 axillary lymph nodes from 35 different subjects. The adult axillary group of lymph nodes was from subjects aged between 18 and 70 years. They were obtained from the Department of Pathology, Shri Sathya Sai Medical College and Research Institute. Care was taken to exclude lymph nodes with clinical or histological evidence of neoplastic and infectious diseases. Fetal lymph nodes were collected from 8 still born fetuses (5 females and 3 males) ranging between 37 and 42 weeks. The fetuses were procured from the Department of Obstetrics and Gynecology after getting informed consent. The study was conducted after obtaining institutional ethical clearance.

**Age distribution:** The samples of the axillary lymph nodes were categorized into three groups:

The fixed nodes were carefully halved with a razor blade in a plane perpendicular to their longer diameter. After proper fixation, the tissue was subjected to routine processing with dehydration in ascending grades of alcohol, clearing in xylene (3 changes), and finally impregnation and embedding in molten paraffin wax; 5 µm thick sections were cut using rotary microtome and the sections were then stained with hematoxylin and eosin. Thickness of the cortex and diameter of the germinal centers were measured using ocular and stage micrometer. Statistical analysis was done by employing a 2-tailed student t-test to compare the parameters described earlier.

**Observation and Results**

**Group I (full-term fetus; 37–42 Weeks)**

**Germinal Center**

Small primary follicles were discernible in the outer cortex and 5 out of the 8 lymph nodes studied elicited corticomedullary differentiation (Fig. 1a). None of the lymphocytic follicles showed evidence of a prominent germinal center (Fig. 1b).

**Deep Cortical Region**

The deep cortical region (diffuse lymphoid tissue) was greatly expanded (Fig. 2a) in all the full-term fetuses and could not be measured using ocular micrometer. The deep cortical regions showed rich vascularity and numerous post capillary venules (Fig. 2b). Aggregations of lymphocytes were particularly found around post capillary venules. Wide trabecular sinuses observed in one of the lymph nodes were found to be communicating with one another (Fig. 3).

**Lipomatous Atrophy**

It was not encountered in any of the 8 fetuses.

**Group II (Young Adult; 18–35 Years)**

**Germinal Center**

The germinal centers of this group were markedly prominent, larger in size and more in number (Fig. 4) when compared with the older group of lymph nodes. Prominent germinal centers with a mean actual diameter of 77 µm were found in all lymph nodes.

**Paracortical/Deep Cortical Region**

The lymph nodes showed well defined deep cortical regions often containing large lymphoblasts and prominent
Fig. 1 Fetal lymph node (38 weeks) showing (a) corticomedullary differentiation and small lymphocytic follicles with no prominent germinal centre in the outer cortex (H&E stain 10X) and (b) lymphotic follicle with no prominent germinal centre (H&E stain 40X).

Fig. 2 Fetal lymph node (37 weeks) showing (a) greatly expanded paracortical regions (regions of diffuse lymphoid tissue) with many post capillary venules (H&E stain 10X) and (b) aggregations of lymphocytes around post capillary venules (H&E stain 40X).

Fig. 3 Fetal lymph node (37 weeks) showing trabecular sinuses communicating with each other.

Lipomatous Atrophy
Interestingly, lipomatous atrophy was encountered in 2 of the 15 lymph nodes (13.3%) of this group (►Fig. 5b).

Group III (Old; 60–70 Years)
Germinal Center
An overall reduction in the number of lymphocytic follicles with germinal centers was observed with an appreciable amount of diffuse lymphoid tissue between the follicles. Moreover, a reduction in the very size of the germinal center itself was noted. The mean actual diameter of the germinal center was 36 µm (►Fig. 6). The diameter of the germinal center in the younger population was significantly greater than in the older group ($p < 0.018$). ►Graph 1 represents the germinal centre diameters of various age groups.

Paracortical/Deep Cortical Region
The mean thickness of the deep cortex was 291 µm (►Fig. 7a). The thickness of the paracortical region in the old was significantly reduced ($p < 0.012$) when compared with the young (423 µm). Thus, signifying an age-related involution.

Lipomatous Atrophy
Highest incidence of lipomatous atrophy was found in this group with 10 out of the 12 lymph nodes studied displaying it. This amounted to a marked 83.3% of the lymph nodes (►Fig. 7b). ►Graph 2 compares the lipomatous atrophy of lymph nodes in different age groups.

endothelial cells in small blood vessels. The thickness of the deep cortex was however reduced when compared with its fetal counterpart. The mean thickness of the deep cortical region was 423 µm (►Fig. 5a).
Discussion

Germinal Center

Of all the parameters examined, the number and size of the germinal centers showed the most impressive age-dependent changes. None of the fetal (37–42 weeks) lymphocytic follicles showed evidence of a prominent germinal center. This was in agreement with the general opinion that germinal centers appear sometime after birth, within the second or third month. There also persisted a general opinion that germinal centers were more frequent in young adults when compared with the old. Tsakraklides et al. observed that germinal centers were more frequent in young adults than the old. The present study confirms this documentation and also adds on that the germinal centers were not only more numerous but also larger in size, with the mean actual diameter being 77 µm. In group III (old), an overall reduction in the number and size of the lymphocytic follicles with germinal centers was noted. The mean actual diameter of the germinal center was reduced to 36 µm. Studies on young and aging mice have demonstrated decreased B responsiveness to antigens with age. Moreover, repeated stimulation with the same antigen leads to dissolution of germinal centers. In addition to that, it is reasonable to assume that fewer and fewer new antigens are encountered with advancing age.

Deep Cortical Region

The fetal axillary lymph nodes displayed greatly expanded deep cortical regions which showed rich vascularity and
numerous post capillary venules. Aggregations of lymphocytes were particularly found around post capillary venules. This finding supports reports of Miller, Herman et al., Soderstrom, Mikata et al., Marchesi et al., and Gowans et al., which highlighted the importance of post capillary venules in recirculation of lymphocytes and the development of deep cortical regions. Fetal lymph nodes showed wide sinuses and in one such lymph node, the trabecular sinuses appeared to be communicating with one another.

**Corticomedullary Differentiation in Fetal Lymph Nodes**

There were different opinions about the time of differentiation of cortex and medulla. Silverstein and Lukes did not find evidence of cortical differentiation in the lymph nodes of fetuses. Tsakraklides et al. had found evidence of lymph nodes showing lymphocytic follicles without deep cortical regions and vice versa. Ehrich found that both lymphocytic follicles and deep cortical regions develop during fetal life, but he believed that the deep cortical regions were derived from expansion and fusion of lymphocytic follicles during the immune response. This seems unlikely in view of the cellular composition of the two areas (B cells in lymphocytic follicles, T cells in deep cortex). Bailey and Weis noted the presence of corticomedullary differentiation around 14 to 16 weeks. Markgraf et al. too documented that the differentiation into cortex and medulla became obvious in the 14th gestational week and that small primary follicles were discernible in the outer cortex in the 20th week. In our present study, small primary follicles were discernible in the outer cortex, and 5 out of the 8 lymph nodes studied elicited corticomedullary differentiation.

The young adult axillary lymph nodes showed well-defined deep cortical regions often containing large lymphoblasts and prominent endothelial cells in small blood vessels. The thickness of the deep cortex was, however, reduced when compared with its fetal counterpart but larger than the old axillary lymph nodes. Thus, an age-related involution of the paracortical region was witnessed in the axillary lymph nodes. Makinodan and Adler stated that the decline in the function of the immune system witnessed in old is primarily due to the inability of the precursor cells of the immune system to proliferate and possibly differentiate efficiently, thus suggesting that the paracortical involution with age was just a reflection of the reduction in the circulating T cell numbers. Nevertheless, further immunohistochemical studies could facilitate in deciphering the immunological functions of the lymph node.

**Lipomatous Atrophy**

No evidence of lipomatous atrophy was encountered in any of the fetal lymph nodes. Interestingly, in this current study, lipomatous atrophy which was thought to be exclusively a finding in older age groups was encountered in 2 of the 15 lymph nodes (13.3%). Hadamitzky et al. performed a study on the age-dependent histoarchitectural changes in superficial inguinal human lymph nodes and had also reported that even young lymph nodes displayed degenerative changes like lipomatosis and fibrosis that are mainly age-related. In both these studies, peripheral lymph nodes (axillary and inguinal) which normally exhibit minimal signs of antigenic stimulation were experimented upon. This could possibly be one of the reasons for encountering a premature degenerative change. Nevertheless, these findings were not as marked as observed in senescence. Replacement of lymphatic parenchyma by fat was the most prominent feature in the older groups with a majority of the samples displaying it.

The clinical feasibility of lymph node injections is now well established not only for vaccinations but also for therapeutic interventions like cancer immunotherapy and immunomodulation for allergy or autoimmune disorders. The advent of these novel procedures has demonstrated the need for an in-depth knowledge of the degenerative processes involving lymph nodes. Evaluation of the lymph nodes for degeneration, before venturing into these procedures could optimize the outcomes of these interventions, improve the patient prognosis and enhance the quality of life.

**Conclusion**

This study underscores the importance of age-related differences in the histoarchitecture of lymph nodes. However, premature degenerative changes like lipomatous atrophy can also be witnessed in the young. Hence, lymph nodes display degenerative changes that are not solely age related. The alterations in the microarchitecture of lymph nodes should be taken into account when dealing with them diagnostically and therapeutically in clinical practice. This study advocates the importance of the evaluation of lymph nodes for degeneration before planning and performing nodal interventions.

**Conflict of Interest**

None.

**References**


**Graph 2** Comparison of lipomatous atrophy of lymph nodes.
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