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IMMUNOHISTOCHEMICAL MAPPING OF HASSALL'S CORPUSCLES

Medicine

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ABSTRACT

Introduction: Present study, proposes immunohistochemical criteria for assessing structural and functional characteristics of Hassall's corpuscles using antibodies against cytokeratin markers in human fetuses.

Methods: Hassall's corpuscles of 30 aborted human fetuses of various gestational ages (12 to 31 weeks) were studied by using CK5, CK8 and CK14 antibodies.

Results: CK8 and CK14 positivity was seen around the periphery, while CK5 positivity was observed both at peripheral and central part of Hassall's corpuscles. Two major growth spurts were observed after 16 and 22 weeks of gestation.

Conclusions: This information will be helpful in enhancing the understanding of extent of similarity between the epidermal component and HCs and in designing effective molecular and cellular methods to treat both skin and immune disorders with the help of progenitor stem cells surrounding HCs within the human fetal thymic tissue.

KEYWORDS

Hassall's corpuscles; Cytokeratin; Fetuses, Thymus, Epithelium

INTRODUCTION

The medulla of normal thymus gland contains characteristic swirled epithelial structures known as Hassall's corpuscles (HCs), the marker of ongoing or recent thymopoiesis (Hale and Markert, 2004). It has been reported that thymus from patients with robust thymopoiesis contained non cystic, non calcified HCs and these were reportedly absent in patients who lacked thymopoiesis (Linder, 1987). Various syndromes related with either absence or paucity of HCs are Digeorge's, Nezelof's etc. Although these bodies have been proposed to act in both maturation of thymocytes and removal of apoptotic cells, still their function remains an enigma (Nishio et al., 2000, Hale and Markert, 2004). Rossi et al. (2006) in their study observed presence of epithelial progenitor stem cells within the thymus. Previous studies have also reported positivity of HCs for stratified-epithelium markers (Laster et al., 1986; Heid et al., 1988; Hale and Markert, 2004). Thus, the present investigation aimed to study the immunohistochemical criteria for assessing various structural and functional characteristics of HCs by using various Cytokeratin (CK) markers.

MATERIALS AND METHODS

30 fetuses of various gestational ages, varying from 12– 31weeks were obtained from the department of Anatomy, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh and Government Medical College and Hospital (GMCH), Chandigarh. Institute committee had given clearance for research work on fetuses. Parental consent was obtained in each case. Fetuses were preserved in 10 % formalin. Thymic tissue sections were taken on special polylysine coated slides and immunohistochemistry was done using Avidin Biotin Complex technique (ABC) technique.

Slides were given three changes of xylene for ten minutes, followed by three changes of graded alcohol for ten minutes each. Endogenous peroxidase was blocked for twenty minutes using solution of Phosphate Buffered Saline solution (PBS) containing hydrogen peroxide (80 ml PBS, 20 ml H_2O_2). After washing in PBS solution, slides were immersed in solution containing Citrate buffer, and placed in the microwave for retrieval of antigen and then washed with 2.5% Bovine Serum Albumin (BSA). Subsequently, slides were incubated at 4˚C overnight with following primary antibodies: Cytokeratin 5 rabbit anti human monoclonal antibody (Cell Marque, USA); Cytokeratin 14 mouse anti human monoclonal antibody (Cell Marque, USA); Cytokeratin 8 mouse anti human monoclonal antibody (Cell Marque, USA).

The slides were washed three times with PBS for five minutes each and further, incubated for two hours at room temperature with secondary antibody (DAKO company, Envision pro). After rinsing with PBS solution, slides were treated with 0.025% 3,3 diaminobenzidine and 0.003% H_2O_2 medium, under room temperature for colour development. After rinsing with distilled water, slides were counterstained with Meyer's Haematoxylin and then mounted with DPX solution, and examined under an Olympus BX53 microscope. Slides were dehydrated with ascending grades of alcohol and mounted. Appropriate negative controls (obtained by omission of the primary antibody) and positive controls were used throughout. Analysis and image documentation was carried out with Prog Res Capture Pro 2.9.01.

i. Evaluation of immunoreactivity - Quantification of data was done by counting the number of CK8, CK5 and CK14 positive cells in ten random high power fields in each slide.
ii. Statistical analysis of the data was performed using SPSS software (version 20; IBM SPSS Inc., IL, USA), by using Student's t test, followed by post hoc Kruskal-Wallis rank test. The data was reported as mean (median) ± standard deviation (and standard error mean). The level of statistical significance was set at <0.05 (two-tailed p-values).
iii. Measurements were done with the help of Prog Res Capture Pro 2.9.01 software (Fig. 1).

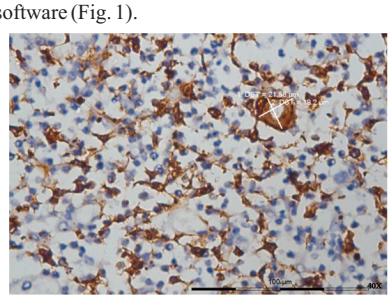

Figure 1

Hassall's corpuscles were observed under Olympus BX53 microscope and measurements were done with the help of Prog Res Capture Pro 2.9.01 software.

RESULTS

i.Time of appearance of Hassall's corpuscles (Fig. 2)

It was observed that Hassall's corpuscles (HCs) appeared at 13 weeks of gestation. At this stage, they were small in size and lacked well defined structural characteristics like whorls of epithelial cells etc.

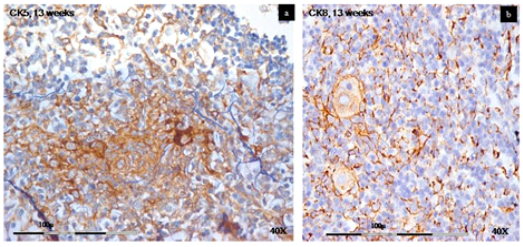

Figure 2

Time of appearance of Hassall's corpuscles (shown by arrows) in human fetal thymic tissue was observed to be 13 weeks as seen on immunolabelling with (a) CK8 and (b) CK5 (Scale bars 100µ).

ii.Development of Hassall's corpuscles (Fig. 3)

With advancing gestational ages, various stages of formation of HCs were observed, which were classified into four types (Raica et al., 2006):

Type I (Juvenile) – One or two hypertrophic cells were observed without any keratinization. This type was seen among 14 – 15 weeks fetuses.

Type II (Premature) – Though keratinization had begun, but still no concentric whorls were seen. It was observed in 15 to 17 weeks fetuses. Types III (Mature) – Cells were seen arranged concentrically to form characteristic whorls. This type was observed in fetuses beyond 16 weeks.

Type IV (Advanced) – Two or three HCs were seen clubbed together giving distorted appearance. These were seen in more than 22 weeks of gestational age.

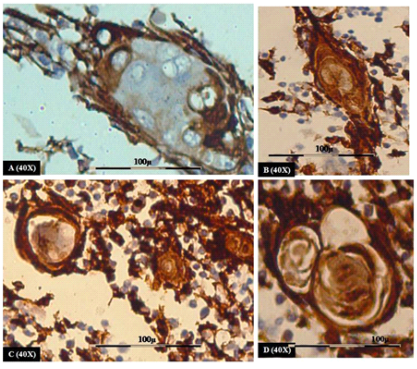

Figure 3

The immune stained slides showed the different stages of development of the Hassall's corpuscles. These stages were classified into four types as follows:

(A) Type I (Juvenile) (B) Type II (Premature) (C) Type III (Mature) (D) Type IV (Advanced) (Scale bars 100µ)

iii. Expression of Cytokeratin (CK) 5, 8 and 14 (Fig. 4)

HCs stained positive with both both CK 5 and CK8 from 13 weeks onwards. CK5 was found to be positive both at the periphery as well as in the centre of HCs, while CK8 positivity was observed in the periphery only. CK14 exhibited entirely different pattern; it showed no expression before 16 weeks but faintly positive expression was seen in one or two cells lying at the periphery at 16 weeks. However, after 17 weeks CK14 staining was found to be intense in the periphery of Hcs.

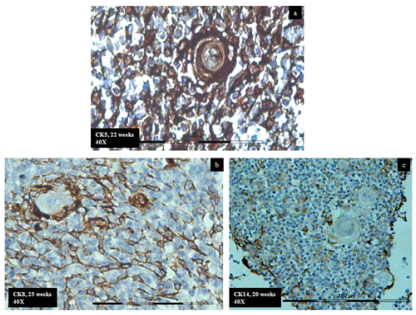

Figure 4

On immunolabelling Hassall' corpuscles with different cytokines, (a) CK5 was found to be positive both at the periphery as well as in the centre of HCs, while (b) CK8 and (c) CK14 positivity was observed in the periphery only (Scale bars 100µ).

iv.Number of Hassall's corpuscles (Fig. 5)

The number of HCs was found to increase with advancing fetal age. They ranged from 1 – 17 HCs per 10 hpf (high power field). Maximum number of corpuscles were observed beyond the age of 22 weeks. Statistically significant ($p<0.05$) increase in the number of HCs was observed after 16 and 22 weeks of gestation.

Length of HCs varied from 8 µ to 900 µ and breadth varied from 12 µ - 1370 µ. We observed variable shapes of HCs like spindle, rocket, spherical, oval, circular, conical and irregular.

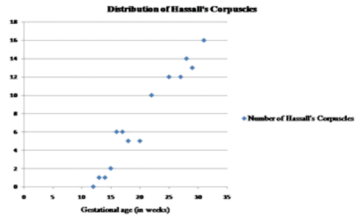

Figure 5

The graph shows the differential distribution pattern of Hassall's corpuscles. Two major growth spurts were observed in the number of Hassall's corpuscles after 16 weeks and 22 weeks of gestation.

DISCUSSION

In this investigation, we present data of morphology, development, cytokeratin expression, distribution and polymorphic features of HCs in human fetal thymuses. It was observed that CK 8 and 14 were expressed in the peripheral regions of HCs. CK8 is a simple epithelial marker, while CK14 is a stratified epithelial marker (Shezen et al., 1995). Thus, simple (CK8) as well as stratified epithelial markers (CK14) were seen around the periphery of HCs. Other studies also reported that the peripheral portion of HCs expressed simple (CK7, 8, 18) and stratified epithelial markers (Laster et al., 1986; Heid et al., 1988; Shezen et al., 1995; Hale and Markert, 2004; Gupta et al., 2016). It has been observed that p63 controls stratified epithelial development in both skin and thymic tissues through fibroblast growth factor receptor 2β (FGFR2β) signaling (Revest ., al., 2001; Rice et al., 2004), and thus *p63* null patients present with defect in epidermal as well as thymic tissue development (Laurikkala et al., 2006; Candy et al., 2007). While the functions of skin and thymus broadly seem quite distinct, but both tissues have primary roles in establishing immunity. Epidermal keratinocytes play a role in activation of immune system by producing cytokines (Swamy et al., 2010; Roberts and Hosley, 2014). Furthermore, the developmental program that helps in formation of HCs from medullary thymic epithelial cells is analogous to that of formation of cornified cells from skin epidermal basal cells (Lobach and Haynes, 1987). These findings suggest similarity between epidermal component of skin and HCs, derived from medullary thymic epithelial cells. This demonstration in human fetal thymus of cytokeratins supports the hypothesis that thymic and epidermal cells undergo similar stages of differentiation (Laster et al., 1986).

Another important finding in the current study was positive expression

of CK5 both at the periphery as well as in the inner part of HCs. CK5 is the marker of basal cells in stratified epithelium (Nagle et al., 1986), thus showing presence of cells similar to basal layer of stratified epithelium within the HCs. Basal cell layer has stem cell properties and is also called as stratum germinativum. Further, CK5 and CK8 are also progenitor stem cell markers (Lai and Jin, 2009) thus showing presence of progenitor stem cells around the periphery as well as inner region of HCs from 13 weeks onwards. This knowledge can be used in treatment of various immunodeficiency diseases of skin and thymic tissue.

In the present study, time of appearance of HCs was found to be at 13th week, which is same as given by Varga et al. (2011), whereas Williams et al. (1995) and Ghali et al. (1980) observed HCs at 10th and 11th weeks, respectively. Regarding distribution of HCs, Liberti et al (1994) and Krishnamurthy et al. (2015) in their studies reported that HCs increased in number with the fetal age, with a significant increase after 16weeks and 20weeks. Our findings also tally with these workers.

We found that the size of HCs varied significantly from very small to very large and variable shapes like cone, tricycle, comma, elongated etc. High rate of such polymorphism in HCs was seen after 28 weeks of gestation in the current study as well as previous studies (Blau et al., 1968; Asghar et al., 2012; Blackburn et al., 2002). The polymorphism observed in HCs can be attributed to their role in negative selection and apoptotis of thymocytes which occurs around 28 weeks, thus leading to increased rate of degeneration and keratinization of thymic epithelial cells which later on anchor to the peripheral regions of HCs resulting in variability in their sizes and shapes.

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