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The TIMP-1 expression in germinal centers of hypertrophied adenoids in children

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ABSTRACT

Objectives: The main aim of this study was to evaluate TIMP-1 expression in germinal centers of hypertrophied adenoids in children on the assumption that it can be treated as a marker of adenoidal tissue function.
Patients and methods: The study involved 54 children undergoing adenoidectomy; divided into three age groups: aged up to 5 years (8 children), 5–10 years (31 children) and over 10 years (15 children). The analyzed material was adenoids removed on the ground of hypertrophy, which caused obstructive symptoms and/or otitis media with effusion onset. Immunohistochemical analyses were carried out using monoclonal mouse antibody (Ab) (Novocastra) directed against human TIMP-1 protein. The presence of TIMP-1 positive lymphocytes within germinal centers and TIMP-1 immunostaining were scored.
Results: The immunohistochemical staining showed the TIMP-1 positive lymphocytes mainly within the mantle zone. There was no statistically significant difference between the mean age of children for TIMP-1 immunoreaction levels. We have not found statistical correlation between the TIMP-1 staining and the clinical status of patients.
Conclusion: It is difficult to interpret our results. Our findings did not demonstrate changes in TIMP-1 expression according to age. This may indicate that the processes of hyperplasia, hypertrophy and atrophy of adenoid are not influenced by age and support our thesis that adenoid involution is rather the effect of changes in the number of lymphoid follicles that changes in them. However there is a need for further observational studies of TIMPs and MMPs in adenoid tissue.

1. Introduction

Apoptosis is considered to be related to morphological and immunological involution of the lymphatic system [1]. It plays a central role during B cell development [2]. Though tonsils are peripheral lymphoid organs, B cells may disappear through the mechanism of apoptosis [1]. The balance between the continual generation of new cells and their elimination maintains B cell homeostasis [3]. In lymphocytes there are at least two independent pathways for initiation of programmed cell death: the tumor necrosis receptor family pathway, and the Bcl-2 family-dependant pathway [4]. The initiation of apoptosis has been correlated in several cell types with aberrant regulation of the cell cycle and proliferation. Apoptotic events commence as the ratio of extracellular matrix degrading proteinases to inhibitors increases [5].

Extracellular matrix (ECM) is involved in tissue homeostasis and in tissue remodeling and destruction associated with inflammatory conditions [6]. It not only provides a structural framework for tissue but also plays an important regulatory role for cell proliferation, apoptosis, migration and differentiation [5]. Two groups of protein, matrix metalloproteinases (MMPs) and their tissue inhibitors, TIMPs, are accepted as being the important factors for maintenance of extracellular matrix homeostasis. TIMPs are secreted by the same types of cells that produce MMPs. The balance between MMPs and their inhibitors is critical in tissue repair and remodeling and in the breakdown and deposition of extracellular matrix [6,7]. There are four confirmed variants of TIMPs in mammals: TIMP-1, -2, -3 and -4 [6,8,9]. TIMP-1 seems to be the most important endogenous inhibitor of matrix metaloproteinases [6]. It is a 28.5-kDa glycoprotein consisting of 184 amino acids in the mature protein; located in the cytoplasm of cells [10] and in the nucleus [11]. TIMP-1 is produced by various kinds of cells and found in every human body fluid examined, suggesting that TIMP-1 is a fundamental and ubiquitous protein in human beings [12].

TIMP-1 is a survival and differentiation factor for B cells. It has been shown to suppress apoptosis of B cells. It does it in the absence of Bcl-2 overexpression. Blc-2 overexpression results in induction of TIMP-1 expression [13].

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The intracellular signaling pathways underlying the anti-apoptotic effect of TIMP-1 remains poorly defined [14] and the probable contribution of extracellular matrix turnover dysregulation to the tonsillar hypertrophy are not still well known [15].

We were unable to find data in the literature regarding the status of metalloproteinases and their inhibitors in children with hypertrophied adenoids.

The main aim of this study was to evaluate TIMP-1 expression in germinal centers of hypertrophied adenoids in children.

2. Materials and methods

The study involved 54 children undergoing adenoidectomy in the Department of Paediatric Otolaryngology Medical University of Bialystok, Poland, in the year 2002; divided into three age groups: aged up to 5 years (8 children), 5–10 years (31 children) and over 10 years (15 children). The analyzed material was adenoids removed on the ground of hypertrophy, which caused obstructive symptoms and/or otitis media with effusion onset. They were assessed prior to the surgery and during the surgical procedure and they obstructed more than half of the choanal diameter. Once removed, the adenoids were fixed in 10% buffered formaldehyde solution.

For immunohistochemical studies we selected representative section from each case of the adenoid. TIMP-1 was assessed using the monoclonal mouse antibody (Ab) (Novocastra; clone 6F6a) at 1:100 dilution. The sections were deparaffinized in xylenes and hydrated through graded alcohols. Antigen unmasking was performed using heat treatment in a microwave oven at 750 W for 7 min in a container with 10 mM sodium citrate buffer, pH 6.0. Sections were allowed to cool in the buffer at room temperature for 30 min and were rinsed in deionized H_20 three times for 2 min each. The endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 20 min. Next the sections were incubated with TIMP-1 antibody for 1 hour at room temperature using staining chamber (The Binding Site, United Kingdom). After rinsing in three changes of PBS, a streptavidin–biotin–peroxidase complex technique was used to reveal antibody–antigen reactions (LSAB kit, Dako, Denmark). Staining was routinely developed using 3,3′-diaminobenzidine as a chromogen (Dako, Denmark). The tissue sections showing a strong positive reaction of cytoplasmic TIMP-1 protein were used as controls. The sections stained with the immunohistochemical technique were counterstained with hematoxylin to visualize cell nuclei, and then dehydrated in alcohol series and xylene and mounted in Canada balsam. Immunostainings were evaluated with the use of light microscopy (20× and 40× objectives). The evaluation of TIMP-1 expression was analyzed in 10 different fields and the mean percentage of cells with positive staining was evaluated. To describe intensity of immunoreaction of TIMP-1 we used first degree (I°) and second-degree (II°) symbols, respectively. When <10% of cells had positive immunostaining it was marked as I°, and II° when more than 10% of them exhibited positive immunostaining.

In each age group, the level of TIMP-1 expression in lymphocytes of lymphoid follicles was compared with the presence of some clinical features, such as accompanied tonsillar hyperplasia, or otitis media with effusion, rhinitis chronica and a history of frequent infections of upper respiratory tract.

The statistical analysis was performed by the SPSS Statistical Package including the following tests: χ^2-test and Spearman correlation coefficient test. The differences for $p < 0.05$ were considered statistically significant. Study protocol was approved by the local ethical committee.

3. Results

Light microscopy examination revealed a typical histological pattern of lymphoid follicle cross-section, with evident germinal center in the middle and mantle zone outside. The immunohistochemical staining showed the TIMP-1 positive lymphocytes mainly within the mantle zone, where they were evaluated (Figs. 1–3).

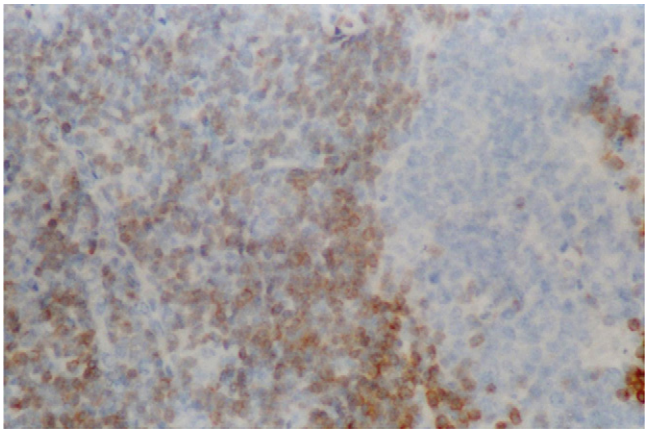

Fig. 1. Detection of TIMP-1 in adenoid tissue sample. I° positive immunostaining 400×.

Among all the examined children (54), TIMP-1 positive staining of I° was found in 42 (77.8%) and II° in 12 (22.2%) cases. There was no statistically significant difference between the mean age of children for TIMP-1 immunoreaction levels; 7.31 ± 2.90 years for I° and 8.75 ± 3.33 years for II° respectively. The percentage of TIMP-1 positive reactions in each group age was 87.5% for I° and 12.5% for II° in aged up to 5 years group; 80.6% for I° and 19.4% for II° in 5–10 years group; 66.7% for I° and 33.3% for II° in over 10 years of age group respectively.

We have not found statistical correlation between the TIMP-1 staining and the clinical status of patients. The results are present in Table 1.

4. Discussion

Cell–matrix interactions have been shown to greatly influence cell survival, and withdrawal of anchorage-dependent cells from their association with the extracellular matrix results in apoptotic cell death [16]. During the past several years, investigators have shown critical role for TIMPs in the regulation of apoptosis [17]. Although the precise mechanism by which TIMPs control cell survival remain undefined, their effect may be mediated by their

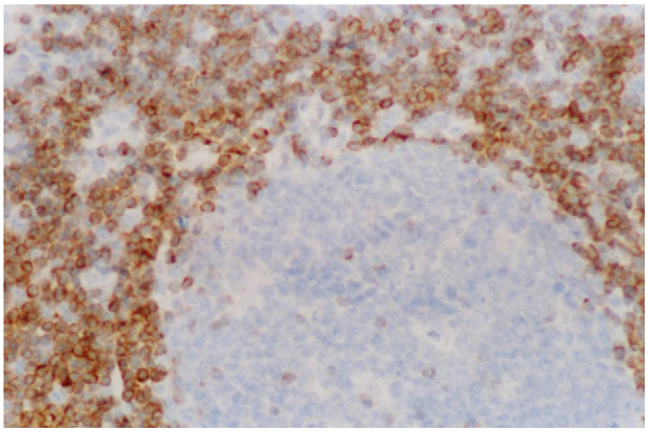

Fig. 2. Detection of TIMP-1 in adenoid tissue sample. II° positive immunostaining 400×.