Original Article

Evaluation of Topical Corticosteroids in Children with Phimosis through Morphological and Immunohistochemical Analyses of the Foreskin

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Abstract

Introduction: Histopathological analysis of the foreskin has become more common in the last two decades. **Objectives:** This study aims to analyze the morphology of the foreskin and determine the effects of topical corticosteroid therapy on this tissue. **Materials and Methods:** We retrospectively evaluated forty foreskin samples from children aged from 2 years to 15 years with phimosis undergoing circumcision at our institution over a 2-year period. In the foreskin samples, we analyzed the elastic fibers (Verhoeff), epidermal thickness (hematoxylin and eosin), and Annexin 1 and Langerhans cells (LCs) (immunohistochemistry). **Results:** In the present study, 18 (45%) patients made use of topical corticosteroids, and 22 (55%) did not, while 4 (10%) had a history of balanoposthitis as previous complication. Forty patients were divided according to the parameter analyzed: with or without previous complication and with or without previous topical corticotherapy. Annexin 1 expression was significantly higher in group with a history of complications when compared with group without complications ($P = 0.024$) and lower in the group of those who used corticosteroids when compared with those who did not used corticosteroids ($P = 0.364$). In the analysis of all samples, the density of mature LCs was significantly higher when compared with immature LCs ($P < 0.0001$). The density of immature LCs was significantly higher in patients without previous complications when compared with group with complications ($P = 0.028$). **Conclusions:** These findings contribute to a better understanding of the histopathological aspects of previous complications and of treatment with corticosteroids in children with phimosis.

Keywords: Annexin 1, corticosteroids, Langerhans cells, phimosis

Introduction

Phimosis is a condition in which the foreskin cannot be retracted over the glans due to a circular band that surrounds and entraps the glans.[1,2] It is estimated that phimosis is present in 96% of children born at term.[3] This condition is clinically classified into physiological and pathological phimosis,[4] and the changes observed in pathological phimosis are usually related to repeated episodes of balanoposthitis. The incidence of pathological phimosis in the pediatric population was estimated at 0.4/1.000 boys per year.[5]

Physiological phimosis normally evolves spontaneously for the complete retraction of the foreskin in 90% of children.[6] Spontaneous resolution is not observed in pathological phimosis, in which the formation of fibrous tissue prevents foreskin retraction.[7] The available therapeutic modalities include the use of topical corticosteroids and surgery in cases of phimosis before adolescence, recurrent urinary infections, severe balanoposthitis, balanitis xerotica obliterans, or vesicoureteral reflux and phimosis.[4] Topical corticosteroids have been used for the treatment of phimosis for at least 2 decades,[3] and their therapeutic effect is attributed to their anti-inflammatory and immunosuppressive actions.[8] However, the mechanisms by which they improve foreskin shrinkage are not clear.[9,10]

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Topical corticosteroids act locally by stimulating the production of annexins, which in turn inhibit the activity of phospholipase A2 and the production of arachidonic acid.[8] Annexins are considered the primary mediators of the inflammatory actions of endogenous and exogenous corticosteroids, especially dexamethasone.[11] In situations when the synthesis of annexins is reduced, the therapeutic action of these steroids is decreased,[12,13] and it has been suggested that the failure of corticosteroid therapy in the treatment of phimosis is due to the decreased expression of annexins.[1]

Regarding the anti-inflammatory effect of topical corticosteroids, their immunosuppressive action is well known, and this action is attributed to the inhibition of phagocytic activity and leukocyte migration.[9] The skin's immune system is composed of chemical substances, such as immunoglobulins, cytokines, and immune complexes, which are produced by specialized cells, i.e., keratinocytes, lymphocytes, antigen-presenting cells called Langerhans cells (LCs), and macrophages.[14] LCs reside in epithelial tissues, such as the skin and mucous membranes.[15] LCs are a type of dendritic cells that interdigitate between epithelial cells, exhibiting functions involving antigen presentation and the stimulation of the T cell response.[16]

The morphology of LCs varies according to their stage of maturation. Mature LCs are characterized by a star-like morphology, showing long, slender cytoplasmic processes that project from the cell body in various directions. Immature LCs present with a rounded morphology and short cytoplasmic processes.[17] The density of LCs in the foreskin is variable, and it is suggested that this is due to stimulation of these cells by external stimuli, either infectious or not.[18] Further, the density of LCs in the foreskin of children with a history of urinary infections may be greater than the density of these cells in the foreskin of healthy adults.[19] Nevertheless, in neonates, LCs are not observed in the inner foreskin, which adheres to the glans; however, due to this sterile environment, there is no exposure to antigens.[20]

Several authors have proposed histopathological analysis of the foreskin. It has been reported that the foreskin requires a large number of elastic fibers to easily expose the glans.[21] Furthermore, there is no consensus in the literature about the histological pattern shown in patients with phimosis or the relationship between clinical history and morphological changes after local topical corticotherapy.[7,22-24] Likewise, the real incidence of local side effects, especially skin atrophy, has not been well defined.[25]

Faced with a lack of studies evaluating the histopathological characteristics of the foreskin in cases of topical treatment with corticosteroids, and given the large number of children undergoing circumcision, the present study was designed to improve our understanding of the changes induced in the foreskin by the use of topical steroids by quantifying elastic fibers, Annexin 1 expression, epidermal thickness, and the density of LCs in the foreskin. In addition, we examined whether failed cases of therapy with topical corticosteroids were caused by low Annexin 1 expression levels. Thus, the results obtained in this study may provide important data on mechanisms involved in the failure of treatment with topical corticosteroids. Our findings also suggest that topical therapy with corticosteroids associated with Annexin 1 expression in the treatment of phimosis should be studied further, which may lead to the development of an anti-inflammatory agent with fewer side effects and a more selective action.

Materials and Methods

Patients gave their informed consent for participation in the research study, and this study was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro on February 12 2005, number 638.

We retrospectively evaluated foreskin samples from forty pediatric patients ranging in age from 2 years to 15 years who underwent surgery for circumcision from December 2005 to December 2007. These samples were filed in the general pathology department, and the surgeries were performed at the Clinical Hospital of the Federal University of Triângulo Mineiro.

The patients were divided as follows: Group 1 consisted of 22 (55%) patients not treated with corticosteroids. In this group, the parents or guardians did not accept medical treatment with corticosteroids; Group 2 consisted of 18 (45%) patients treated with topical corticosteroids. In this group, three patients had resolution of phimosis after corticosteroid therapy, and the indication for circumcision was poor hygiene of the glans.

Clinical data were taken from the records of the patients. On the basis of the medical records, we evaluated complications related to phimosis. In patients treated with topical steroids, the standard choice was 0.05% betamethasone valerate in combination with hyaluronidase (ointment) used twice a day for 2 months.

Each foreskin sample was divided into three distinct areas, i.e., the proximal, middle, and distal foreskin; thus, three blade samples represented each case (A, B, and C, respectively). The histological sections were stained with hematoxylin, eosin, and Verhoeff. Morphometric analysis of epidermal thickness was performed by capturing images of fields using a video camera coupled to a common light microscope. These images were analyzed through the interactive system image analyzer of ImageJ.

In the analysis of epidermal thickness, the entire length of the sections was captured and quantified. A total of 15 fields in each fragment were captured. Epidermal thickness was expressed in μm. We used a ×20 objective (final magnification of ×800).

Elastic fiber analysis was performed on slides stained with Verhoeff using a ×20 objective with a final magnification of ×800. Fragment B was used to measure the percentage of elastic fibers because this area is narrower in patients

with phimosis. We analyzed 15 fields in each case. The obtained images were analyzed using an image analyzer system (KS-300, Kontron Zeiss, Augsburg, Germany).

An anti-CD34 primary antibody (Novocastra, 1/100) was used to quantitate the expression of Annexin 1 in foreskin tissue, and an anti-S100 antibody (Dako, 1/400) was used to identify LCs. For the analysis of Annexin 1 expression, we used Leica QWin Plus. The positively stained cells were counted and classified into mature and immature LCs according to their morphology. We analyzed thirty fields in each section.

To calculate the density of LCs in each case, we obtained the average number of LCs in five fields examined under a microscope. Using ImageJ, the epidermis was considered to be a polygon (shape), and the result obtained was transformed into 2 μm and then into 2 mm.

The representativeness of the number of cases in each group was verified in accordance with Motulsky, and in all analyses performed in this study, it was found that the number of cases was representative.[26]

For statistical analysis, an electronic spreadsheet was prepared. The data were analyzed using SigmaStat software version 2.0. (Systat Software, Inc., San Jose, CA, USA). The quantitative variables were tested using the Kolmogorov–Smirnov test to verify whether the distribution was normal. In cases with a normal distribution and similar variances, a Student's *t*-test (*t*) was used for comparisons between two groups, and an analysis of variance (*F*) was used for comparisons between three groups. In this case, the results were expressed as mean ± standard deviation. In cases with a nonnormal distribution, the Mann–Whitney (*T*) test was used to compare two groups, and the Kruskal–Wallis (*H*) test was used to compare three groups. In this situation, the results were expressed as median (minimum–maximum). Differences were considered statistically significant at $P < 0.05$. Graphs were prepared with GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). The same researcher examined all histological samples in a blinded fashion.

Results

A positive but not significant correlation was found between the percentage of elastic fibers and the age of the patients ($P = 0.394$). No significant correlation was found when comparing the percentage of elastic fibers in relation to previous complications, use of topical corticosteroids, and the therapeutic response [Figure 1a and Table 1].

The expression of Annexin 1 was significantly higher in patients who had previous clinical complications ($P = 0.024$) [Figure 1c and Table 1]. In cases with previous complications, the expression of Annexin 1 was significantly higher in the distal foreskin ($P = 0.032$). In the other regions, that is, the medium and distal foreskin, the expression of Annexin 1 was higher, but this difference was not significant. The expression of Annexin 1 was lower in the group that used corticosteroids, but this difference was not significant. When comparing the expression of Annexin 1 and the therapeutic response, lower expression levels of Annexin 1 were observed in the group who used corticosteroids and achieved a therapeutic response; however, this difference was not significant [Table 1].

The thickness of the epidermis was higher in the group that had previous complications; however, this difference was not statistically significant ($P = 0.803$) [Table 1], but it was significantly higher in the distal foreskin ($P = 0.044$) [Figure 1b]. The average thickness of the epidermis was higher in the foreskin of children who had used corticosteroids; however, this difference was not statistically significant ($P = 0.718$). The thickness of the epidermis was higher in the group that had used corticosteroids and had a clinical response, but this difference was not significant [Table 1].

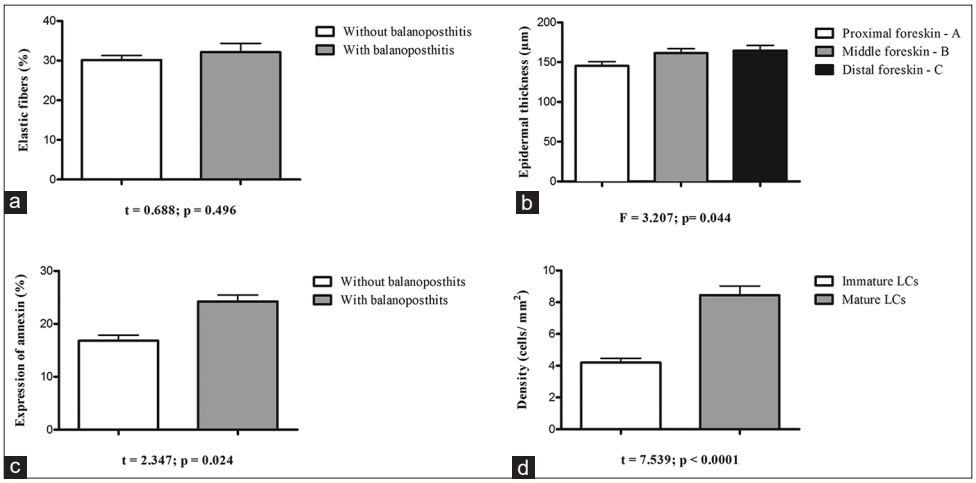

Figure 1: Analysis of the foreskin in pediatric patients undergoing surgery for circumcision. (a) Comparison of the number of elastic fibers between groups with and without previous complications (balanoposthitis). (b) Comparison of the epidermal thickness in three areas of the foreskin. Significant differences: Middle foreskin versus other groups. (c) Comparison of the amount of Annexin between groups with and without previous complications (balanoposthitis). (d) Density of mature Langerhans cells and immature Langerhans cells in samples of foreskin. The error bars represent the standard deviation

Table 1: Morphological and Immunohistochemical Analyses of the Foreskin in Children with Phimosis

Groups	*n* (%)	Elastic fibers (%), Mean±SD	Annexin (%), Mean±SD	Epidermal thickness (μm), Mean±SD	LCs density (cells/mm²), Mean±SD
Complications					
No	36 (90)	29.63±7.09	16.80±6.29	151.58±27.24	12.82±4.45
Yes	4 (10)	32.13±4.42	24.23±2.43	155.23±31.49	11.13±2.97
Total	40 (100)				
t, *P*		0.688, 0.496	2.347, 0.024	0.251, 0.803	0.736, 0.466
Groups	***n* (%)**	**Elastic fibers (%), Mean±SD**	**Annexin (%), Mean±SD**	**Epidermal thickness (μm), Mean±SD**	**LCs density (cells/mm²), median (minimum-maximum)**
Topical corticotherapy					
No	22 (55)	30.24±6.95	18.38±6.31	150.46±27.10	11.30 (9.81-15.03)
Yes	18 (45)	29.45±6.94	16.53±6.42	153.74±29.02	11,15 (10.01-14.98)
Total	40 (100)				
t, *P*		0.360, 0.721	0.919, 0.364	0.364, 0.718	331.000, 0.810
Therapeutic response					
No treatment	22 (55)	30.24±6.95	18.38±6.31	150.46±27.10	11.30 (9.81-15.03)
No response	15 (37.5)	29.93±6.46	16.86±6.35	151.81±29.30	11.07 (9.27-14.83)
Improve	3 (2.5)	27.05±10.35	14.83±7.90	162.76±31.76	11.15 (1063-14.64)
Total	40 (100)				
F, *P*		0.275, 0.761	0.539, 0.588	0.511, 0.604	H=0.244 *P*=0.885

LC: Langerhans cells, DP: standard deviation

The density of mature LCs in all forty foreskin samples was significantly higher than that of immature cells ($P < 0.0001$) [Figure 1d]. The density of immature LCs was significantly higher in patients without previous complications ($P = 0.028$). There was no significant difference in the density of LCs in children who used topical corticosteroids [Table 1].

Discussion

Histopathological analysis of the foreskin has become more common in the last two decades, and several authors have reported their importance in the identification of pathological processes that affect this region.[7,10,18] In addition, advances in histological techniques, especially in immunohistochemistry, have allowed us to understand important steps in biological processes related to local immunity.[19] However, other authors suggested that histopathological analysis is only required in cases in which topical corticosteroid therapy fails.[27]

The present study demonstrated a positive but not significant correlation between the number of elastic fibers and age. The low rate of renewal of elastic fiber components causes a decrease in the number of elastic fibers with age.[28] The percentage of elastic fibers was smaller in patients who had received corticosteroids and demonstrated a therapeutic response, but this difference was not significant. Topical corticosteroids cause a change in extracellular matrix (ECM) components by inhibiting the synthesis of glycosaminoglycans by dermal fibroblasts and the synthesis of collagen.[9] The smaller number of elastic fibers in the foreskin and phimosis in patients undergoing treatment with corticosteroids is similar to what happens during the healing process and may be related to the difficulty of exposing the glans.[7]

The thickness of the epidermis was greater in patients with a history of balanoposthitis and significantly higher in the distal foreskin. This is in agreement with previous studies, since the greater thickness of the epidermis of the foreskin has been described in patients with recurrent urinary tract infections.[19] However, in our patients, no episodes of urinary infections were observed. Other infectious processes, such as balanoposthitis, can be related to the increased thickness of the epidermis of the foreskin. There was no significant difference in epidermal thickness in patients treated with topical steroids. Local skin atrophy is the most common side effect of corticotherapy.[29] However, this change affects the epidermis and dermis and is reversible after treatment is stopped.[30] Despite the fact that corticosteroids were applied for 8 weeks, we did not detect histological signs of cutaneous atrophy. Thus, this confirms the safety of this therapy in pediatric patients with phimosis.

The expression of Annexin 1 was significantly higher in the group of patients who had previous clinical complications. The expression of annexins is reportedly high as long as the inflammatory process continues.[31] The expression of Annexin 1 in patients who had used corticosteroids was lower, but not significantly so. The use of topical steroids alters the expression of annexins, increasing their expression approximately 3 h after use and returning it to baseline after 18 h.[32] However, according to our results, the reduced expression of Annexin 1 compared to the group receiving topical corticosteroids can

be explained by the time between the end of topical therapy and surgery.

There was a nonsignificant positive correlation between the density of LCs and age, between mature LCs and age and a nonsignificant negative correlation between immature LCs and age. The density of mature LCs was significantly higher than the density of immature LCs, independent of a history of balanoposthitis. This is in agreement with previous studies, since the density of LCs depends on external inducement,[33] and these stimuli occur gradually. We understand that balanoposthitis does not represent a sufficient condition for the recruitment of LCs, especially mature LCs. The use of topical corticosteroids did not have an influence on the distribution of mature and immature LCs. Steroids induce their immunosuppressive effects through the inhibition of leukocyte migration and phagocytosis; however, their actions are also related to their anti-inflammatory properties.[34]

One of the limitations of this study is that although the number of cases is considered representative according to the analysis proposed by Motulsky,[26] a relatively low number of patients could be included in this study. We might find more significant results if the number of cases was higher. Moreover, it would be very interesting if we could include a control group with samples from patients without phimosis who were not subjected to topical corticosteroid therapy. This group could help explain many aspects of the present study, especially the analysis of the effect of topical steroids on elastic fibers; however, for ethical reasons, this is not possible.

Although they are important for foreskin retraction, the elastic fibers are not the only responsible factor in the etiology of phimosis, and they do not undergo quantitative changes after the use of topical steroids. Cutaneous atrophy is an uncommon side effect in children undergoing topical steroid treatment. In this study, we did not identify this side effect, but it should be kept in mind. We demonstrated that the increased expression of Annexin 1 in the foreskin of children is a sensitive indicator of a history of balanoposthitis, and this complication must be considered an important indication for circumcision before the development of pathological phimosis. The population of LCs is not altered with the use of topical corticosteroids, and mature LCs are predominant in children's foreskins. These data seem to represent new information about this theme that does not appear to have been reported in the literature until this moment.

Conclusions

The histopathological study of the foreskin of children undergoing circumcision provides important data on mechanisms involved in the failure of treatment with topical corticosteroids. We recommend that this practice should be used routinely in children undergoing surgery. This analysis of the foreskin after the surgical procedure can provide new information about the pathogenesis of phimosis, concomitant lesions, and their response to treatment, which may contribute to the development of new propaedeutic and therapeutic approaches.

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Conflicts of interest

There are no conflicts of interest.

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