Journal of Paediatric Surgeons of Bangladesh (2011) Vol. 2 (1): 17-21
Official organ of the Association of Paediatric Surgeons of Bangladesh

Journal of Paediatric Surgeons of Bangladesh

Original Article

HISTOLOGICAL ANALYSIS OF VASCULAR AND COLLAGEN TISSUES IN THE VENTRAL AND CORRESPONDING DORSAL SKIN OF MID-PENILE HYPOSPADIAC PENIS AT THE MEATAL AND CORONAL LEVELS: A COMPARATIVE STUDY

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Abstract

Background: Hypospadias where surgery is the only option to correct this birth defect which is usually associated with post-operative urethrocutaneous fistula formation even in best hands. The site of fistula is mostly at coronal level (80%) and less common at meatal level. Many factors are supposed to be responsible for causation of this fistula, important one is the developmentally defective ventral penile skin at and / or distal to the urethral meatus where some local healing factors like vascular and collagen tissues are insufficient. Objective: To determine histologically whether the ventral skin of hypospadiac penis at the meatal and coronal levels differ from the corresponding dorsal skin levels in terms of vascular and collagen tissue. Study design: Observational comparative study. Place of study: (1) Department of Paediatric Surgery, Bangabandhu Sheikh Mujib Medical University, Dhaka. Period of study: December 2008 to 15 May 2010. Materials & Methods: A total of 10 patients with mid-penile hypospadias without chordee were included in this study. Representative skin tissues were collected from ventral skin and corresponding dorsal skin of the meatal and coronal levels of every patient. Haematoxylin-eosin stained slides were prepared from each tissue specimen and were examined under microscope to determine the differences in blood vessels and collagen tissues distribution. Paired 't' test was used to test the significance of differences. Results: Significantly lower proportion of sectioned blood vessels was found in the ventral skin than in the dorsal skin at the coronal level. The proportion of sectioned collagen fibre bundles was also significantly lower in the dorsal skin than in the ventral skin at the coronal level. Conclusion: This study reveals difference in distribution of blood vessels and collagen fibre bundle in the ventral skin in comparison with corresponding dorsal skin of hypospadiac penis at meatal and coronal levels.

Key words: Hypospadias, urethrocutaneous fistula, meatal level, coronal level, vascular tissue, collagen tissue.

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Introduction

Hypospadias is defined as an arrest in the normal development of urethra characterized by absence of urethral meatus at the tip of the glans but it is present at the undersurface of the penis along the shaft, even at the perineum[1]. It occurs 1 in 350 live-births[11]. Duckett in 1996 classified hypospadias according to the location of the urethral meatus as (1). Anterior group (49%) comprising the glanular, sub-coronal and distal penile varieties (2). Middle group (21%) comprising only mid-penile variety and (3). Posterior group (30%) comprising the proximal penile, peno-scrotal, scrotal and perineal varieties[7].

This developmental urethral arrest gives cosmetically an abnormal appearance of the penis. The prepusal skin is excess dorsally giving it's hooded appearance but ventrally it is deficient. The urethra distal to the meatus is replaced by urethral plate, the skin is thin and tightly adherent to the underlying plate. But whole of the dorsal skin and ventral skin, proximal to the meatus are developmentally normal[10].

Surgery is the only treatment option. The timing for surgery varies from surgeon to surgeon, but it is better to do between 6 and 15 months[15].The complications are much higher after hypospadias surgery than any

other reconstructive procedure[3]. Among the complications, urethrocutaneous fistula is the most frustrating and difficult to manage. To overcome this post-operative complication, more than 300 different surgical techniques have been developed[2]. Despite the best efforts and techniques, the complication like urethrocutaneous fistula still occurs in a significant number of patients[4].

In one study, it has been shown that the rate of fistula formation after initial surgery is 5 to 44 % [9]. Several authors found in their studies that, around 80% of fistula occurs at the corona level[13]. The next commonest site of fistula is the beginning of the neourethra, i.e, at the level of the hypospadiac meatus[8].

Why does fistula occur even in the best hands? Why does it occur at the corona level and at the beginning of neourethra? Are they representing weak points? Histological analysis of both ventral and dorsal skin shows that at the site of the fistula, some important local healing factors are deficient[10]. It is assumed that deficiencies are more marked at the coronal level than at meatal level.

The supply of blood to a wound area is a crucial factor for its healing. The collagen content of that area is also an important factor for healing. In one histological study it was found that extensive amount of blood vessels were present at the urethral plate, but no difference of collagen content between the ventral and dorsal skin of hypospadiac penis[6]. In another study it was commented that despite the clinical use of urethral plate to form neourethra, its histological characteristics have not been well understood[12]. Another hypospadiologist reviewed the literature regarding histological findings and made the statement that data about the difference in vascularity between hypospadiac and normal penis were lacking [14]. Thus, at present there seems to be a lot of confusions regarding the histological findings of hypospadiac penile skin among the authors who have worked in this field. Therefore, it was felt that any study on this possible histological aspect of the failure of hypospadias repair might be worthwhile both for understanding the reason and developing remedial measures.

So the present study was designed to find out the possible histological differences between the ventral and corresponding dorsal skin of hypospadiac penis in terms of the vascularity and the amount of collagen fibres in two weak points-at coronal level and at meatal level.

Details of the Method

During surgery of the primary hypospadias of mid-penile variety without chordee, representative tissues (2mm x 2mm) from each of the six selected sites were collected from every patient. Four from the ventral aspect(two from the meatal level represented by Vmr and Vml and two from coronal level represented by Vcr and Vcl) and two from the dorsal aspect of the penis, of meatal level(Dm) and of coronal level(Dc). Ten percent formalin was used as preservative. Each piece of tissue was numbered in a single blind method by the supervisor of the research. In the laboratory, each tissue piece was embedded in paraffin and was sectioned by microtome. The slides were stained with hematoxylin-eosin stain and were examined under ordinary light microscope (OLYMPUS). A pencil outline of the slide was drawn on a white paper using a periscopic drawing tube attached to the microscope. Five fields from every slide were selected by drawing arbitrary circles - four from four corners and one from the centre. Each field was examined using a 40 x objective and 10 x eyepiece (high - power field) for sectioned blood vessels and sectioned collagen fibre bundles. The blood vessels were seen as luminal structure lined by endothelial cells and collagen fibre bundles were seen as thick and irregular pink colored structures. For counting collagen fibre bundles and blood vessels, a special circular transparent counting sheet, 0.95 cm in diameter, was devised with the help of a graphic designer. It contained 33 points at equal distances on parallel line printed positive photo film (Fig.-1). This counting sheet was fitted in the eye-piece of the microscope so that the 33 points could be viewed as superimposed over a particular microscopic field of a tissue section. Each point superimposed on a sectioned collagen fibre bundle or a blood vessel was counted. The sectioned blood vessels and sectioned collagen fibre bundle occupying the microscopic field outside these 33 points were not counted. In this way, the number of points falling on blood vessels and the number of points falling on collagen fibre bundles were counted separately from each field of a slide. The counting was recorded on a special counting row. Then the individual value of each variable from each field was multiplied by a factor of 3.03 (for 33 x 3.03 = 99.99, almost 100) to make the variables as proportion of 100. The results of the five fields after multiplication were arranged serially in the table both for blood vessels and for collagen fibre bundles. Finally, the mean of the five values was calculated separately for each variable. These means represented the relative proportion of sectioned blood vessels or sectioned collagen fibre bundles per high-power field for the particular penile site of a particular patient. The ventral values Vmr and Vml were averaged to get the ventral skin meatal level (Vm) value. Similarly, the Vcr and Vcl values were averaged to get the ventral skin coronal level (Vc) value. Using the paired ‘t’test, the results were compared as Dm vs Vm, Dc vs Vc, Vm vs Vc and Dm vs Dc.

Results

A total of 10 patients were included in this study with an average age of 6.05 years (range 1.5 years to 12 years). Majority (60%) of the patients were in the 1-5 years age group.

The results regarding the proportions of sectioned blood vessels and sectioned collagen fibre bundles are shown in the following Tables.

Table I shows the proportions of sectioned blood vessels and collagen fibre bundles in different sites of the ventral and dorsal penile skin of the 10 patients. The mean values of the ten patients are also shown.

The comparison of the proportion of sectioned blood vessels between the dorsal skin at the coronal level and ventral skin at the coronal level is shown in Table II. The proportion of sectioned blood vessels was significantly lower ($P < 0.01$) in the ventral skin than that in the dorsal skin.

The comparison of the proportion of sectioned collagen fibre bundles between the dorsal skin at the coronal level and ventral skin at the coronal level is shown in Table III. The proportion of sectioned collagen fibre bundles was significantly lowers ($P < 0.05$) in the dorsal skin than that in the ventral skin.

In this way, the two variables were compared at different levels and statistical results were find out. Statistically significant difference was observed in case of sectioned blood vessels at the coronal level of ventral and dorsal skin, in case of sectioned collagen fibre bundles at the coronal level of dorsal and ventral skin and in the dorsal skin at the coronal and meatal levels.

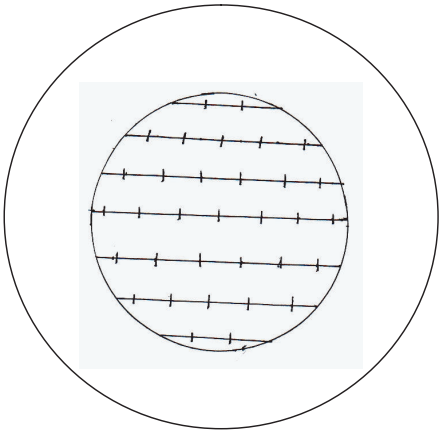

Fig.-1: *Diagram of the special counting sheet carrying 33 points at equal distance on positive photo film (0.95 cm in diameter) put in the eye-piece of the microscope.*

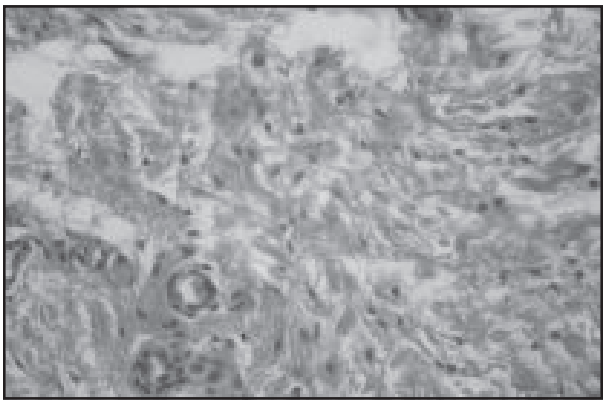

Fig.-2: *Shows a sample photomicrograph showing a representative section of ventral skin at the meatal level (Vm). Three blood vessels are seen on the left part of this section. Pink collagen fibre bundles are irregularly and densely arranged.*

Table-I

Proportions of sectioned blood vessels and collagen fibre bundles in different sites of the ventral and dorsal penile skin of the 10 patients.

Patient no.	Proportion in ventral skin (%)				Proportion in dorsal skin (%)			
	Ventral skin meatal level (Vm)		Ventral skin coronal level (Vc)		Dorsal skin meatal level (Dm)		Dorsal skin coronal level (Dc)	
	BV	Collagen	BV	Collagen	BV	Collagen	BV	Collagen
1	9.09	75.44	6.05	78.17	7.27	72.72	7.87	77.56
2	8.18	76.96	6.96	81.20	4.24	77.56	6.66	77.56
3	7.27	76.96	8.78	82.71	8.48	80.59	9.69	75.14
4	9.69	76.35	6.66	76.35	4.84	78.78	12.72	72.11
5	6.05	78.77	4.84	78.17	10.90	72.11	12.12	72.72
6	7.57	74.83	4.54	69.68	12.72	78.78	6.66	70.90
7	7.87	81.80	7.87	76.65	7.27	81.81	7.27	79.38
8	3.33	80.09	5.14	78.47	7.87	80.59	8.48	78.17
9	5.45	76.95	5.75	79.08	9.09	80.59	7.87	73.32
10	3.43	72.92	3.53	71.81	6.06	83.02	7.27	67.26
Mean	6.79	77.10	6.01	77.22	7.87	78.65	8.66	74.41

BV: Blood vessel.

Table-II

Comparison of the proportion of sectioned blood vessels between the dorsal skin at the coronal level and ventral skin at the coronal level.

Statistical measure	Proportion in dorsal skin coronal level (Dc)	Proportion in ventral skin coronal level (Vc)	P value
Range	6.66 –12.72%	3.53 – 8.78%	0.009**
Mean + SD	8.66 + 2.17%	6.01 + 1.59%	
0.21NS			

Discussion

As shown in the results, the mean proportion of sectioned blood vessels at the meatal level of the ventral skin was lower than that of the corresponding dorsal skin (6.79% vs 7.89%). But the difference was statistically not significant. On the other hand, the proportion of sectioned blood vessels of the ventral skin at the coronal level was significantly lower than that of the corresponding dorsal skin (6.01% vs 8.66%).

When the proportion of sectioned blood vessels was compared between the ventral skin at meatal level and that at the coronal level, it was found that there was a tendency of a lower value at the coronal level than at the meatal level (6.01% vs 6.79%), although the difference did not reach statistical significance. The fistula also occurs in the ventral skin at the meatal level, i.e. at the beginning of the neourethra as it is less vascular than the dorsal skin at the same level[8]. But the rate of fistula formation is much lower than that at the coronal level.

Of the final four penile sites examined (ventral meatal, ventral coronal, dorsal meatal and dorsal coronal), the lowest mean value (6.01%) was found in the ventral skin at the coronal level. It has been found that most (80%) of the fistula occurs at the coronal level[13]. This may be due to vascular insufficiency as frenular artery is constantly missing in the hypospadiac penis[5].

The mean proportion of sectioned collagen fibre bundles at the meatal level of ventral skin was lower than that of the corresponding dorsal skin (77.10% vs 78.65%). But statistically the difference was not significant. On the contrary, the proportion of sectioned collagen fibre bundles in the dorsal skin at the coronal level was significantly lower than that in the corresponding ventral skin (74.41% vs 77.22%). In the dorsal skin, the coronal level also had a significantly lower proportion of collagen fibre bundles than the dorsal meatal level. Thus, of the four sites of penile skin examined, the lowest mean value (74.41%) was found in the dorsal skin at the coronal level.

Conclusion

The present study dealt with relative proportions of sectioned blood vessels and collagen fibre bundles rather than absolute counts. Knowing the absolute counts of blood vessels and collagen fibre bundles are very important before making any final comment on histological differences between ventral and dorsal skin of hypospadiac penis. This should be kept in mind while evaluating vascular and collagen tissue status of hypospadiac penile skin. However, the results of the present study may be considered as useful in setting a platform for further studies that may lead to solving the problem of fistula formation after correction of hypospadias.

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