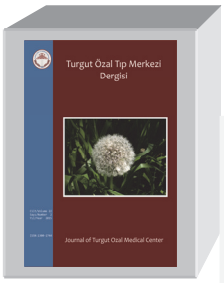

ORİJİNAL MAKALE/ORIGINAL ARTICLE
J Turgut Ozal Med Cent 2015:22(2):81-5

Journal of Turgut Ozal Medical Center
www.jtomc.org

Epidermal Growth Factor Receptor Expression in the Foreskin of Hypospadias Patients

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Abstract
Objectives: The etiology of hypospadias is often obscured and multifactorial. Epidermal growth factor (EGF) accelerates lung and intestinal maturation, promotes cell proliferation, regulates tissue differentiation, and modulates organogenesis and it plays an essential role in the normal regeneration and healing of epithelial surfaces. Inadequate EGFR expression has been reported in periüretral skin.
The aim of our study is to compare the EGFR expression in the dorsal foreskin of children with hypospadias and in foreskin of children undergoing circumcision.
Materials and Methods: The foreskins of 7 healthy children (obtained during circumcision) and 7 children with hypospadias were stained for epidermal growth factor receptor (EGFR) expression.
Results: The EGFR expression in the epidermis, smooth muscle, stroma, blood vessels, and fibroblasts of prepuces were not significantly different between the groups ($p>0,05$). But the number of the mast cells were increased in the hypospadias group ($p<0,05$).
Discussion: EGFR expression did not differ between the dorsal foreskins of children with hypospadias and foreskins of children undergoing circumcision. But the numbers of mast cells were increased in the foreskins of children with hypospadias. Further cellular studies are required to uncover the mechanism underlying the increase in mast cell number.
Key Words: Hypospadias; EGF; EGFR; Mast Cell.

Hipospadias Hastalarının Sünnet Derisinde Epidermal Büyüme Faktörü Reseptörünün Ekspresyonu

Özet
Amaç: Hipospadiasın etyolojisi genellikle belirsiz ve multifaktöriyeldir. Epidermal Büyüme Faktörü (EGF, Epidermal Growth Factor) akciğer ve barsak matürasyonunu hızlandırır, hücre proliferasyonunu kolaylaştırır, doku farklılaşmasını ve onkojenezi düzenler, epitel yüzeylerinin normal rejenerasyonu ve iyileşmesinde çok önemli rol oynar. Periüretral deride yetersiz EGF ekspresyonu bildirilmiştir.
Çalışmamızın amacı hipospadiaslı çocukların sünnet derisindeki ve sünnet olan çocukların sünnet derisindeki EGFR ekspresyonlarını karşılaştırmaktı.
Gereç ve Yöntem: Yedi sağlıklı çocuğun (sünnet sırasında alınmıştır) ve 7 hipospadiaslı çocuğun sünnet derileri epidermal büyüme faktörü reseptörü (EGFR) ekspresyonunun tespiti için boyanmıştır.
Bulgular: Gruplar arasında sünnet derilerinin epidermisinde, düz kasında, stromasında, kan damarlarında ve fibroblastlarındaki EGFR ekspresyonu açısından anlamlı fark yoktu ($p>0.05$). Ancak hipospadias grubunda mast hücrelerinin sayısı artmıştı ($p<0.05$).
Sonuç: Hipospadiaslı çocukların sünnet derisindeki ve sünnet olan çocukların sünnet derisindeki EGFR ekspresyonu farklı değildi. Fakat hipospadiaslı çocukların sünnet derilerinde mast hücrelerinin sayısı artmıştı. Mast hücrelerinin sayısındaki artışın altında yatan mekanizmaları açıklığa kavuşturmak için daha ileri hücresel çalışmalara gerek vardır.
Anahtar Kelimeler: Hipospadias; EGF; EGFR; Mast Hücresi.

INTRODUCTION

Hypospadias is a common congenital abnormality of the male genitalia (1). It occurs in approximately three to five per 1000 births (2). The etiology of hypospadias is often obscured. Efforts to define a clear etiology have been unsuccessful. The etiology of many hypospadias is often assumed to be multifactorial including genetic impairment, or inability to convert testosteron into dihydrotestosterone (DHT), environmental factors, age of the mother, improper androgen receptor signalling, prenatal exposure to progestins, or combined progestins and estrogens (3-5). EGF is a potent mitogen for cells of mesodermal and ectodermal origin (6, 7). EGF accelerates lung and intestinal maturation before birth and in newborn mammals (8). EGF promotes cell proliferation, regulates tissue differentiation, modulates organogenesis, and plays an essential role in the normal regeneration and healing of epithelial surfaces, including respiratory, gastrointestinal, genitourinary, and skin structures (8-10). EGFR regulates multiple facets of cutaneous wound healing, including inflammation, wound contraction, proliferation, migration, and angiogenesis (11). During development of the embryonic testis, somatic and germ cell differentiation and growth are dependent on the expression and action of paracrine growth factors (12, 13). EGF and EGFR are

important paracrine and/or autocrine regulators of spermatogenesis in the bovine (14). As EGF plays an important role in the maturation of various organs before birth, there is a possibility for EGF receptors to participate in the etiology of hypospadias. Publications about this hypothesis are scarce. In this study, we aimed to compare the EGFR expressions in foreskins of children undergoing circumcision and children with hypospadias.

MATERIAL AND METHODS

Inonu University ethics commitee approved this study. We obtained the consent of all our patients. The prepuces from 14 children undergoing circumcision (median age 31,1±9,7 months) and hypospadias surgery (median age 30,4±7,63 months) were examined. Seven patients had subcoronal or distal penile hypospadias. Only non-severe (distal) hypospadias patients were included in the study and hypospadias was the only malformation in every patient. All patients with hypospadias were undergoing their first surgical procedure; we performed the Mathieu procedure. After the surgery, prepuces were fixed in 10% formalin for immunohistochemical examination.

Pathology

Immunohistochemistry: The tissues were fixed in 10% formalin for 24 hrs. Formalin-fixed and paraffin embedded specimens were cut into 4 μm sections, deparaffinized in xylene, and rehydrated in phosphate-buffered saline. The sections stained with immunohistochemistry based on standard streptavidin-biotin peroxidase method (Labvision, Anti-polyvalant, HRP, Westinghouse, USA). Endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide for 8 mins. The sections were stained with primary monoclonal antibodies against EGFR Ab-21 (Labvision, Neomarkers, Westinghouse, USA). The sections were counterstained in Mayer's haematoxylene and then mounted. They were examined by using a Leica DFC 280 light microscope and Leica Q Win Plus Image Analysis System (Leica Micros Imaging Solutions Ltd; Cambridge, UK). The cytoplasmic expression of the EGFR was defined positive. The EGFR staining was scored by a blinded evaluator as follows: unstained (0), weak staining (1), and strong staining (2).

The EGFR expression in the epidermis, smooth muscle, stroma, blood vessels, and fibroblasts were examined in prepuces. We noted an increase in the number of inflammatory cells in prepuces from the hypospadias patients. Therefore, to determine the type of the mononuclear cells, we performed histochemical (dominici and toluidin blue for mast cells) and immunohistochemical (CD 68 for macrophages) analysis. The number of the positively stained mast cells was counted in ten fields for each section.

Statistical analysis

Values are presented as means ± standard deviation (SD). The differences between the groups were evaluated by Mann-Whitney U-test and were considered significant when they reached $P<0.05$. The statistical analysis was performed using the statistical software SPSS 10.0 (SPSS, Chicago, IL, USA).

RESULTS

The foreskin of circumcision patients stratified squamous keratinized epithelium (epidermis) and dermis containing sweat glands were present in the examination (Figure 1). Figure 2 shows the EGFR expression in the smooth muscle, endothelial cells, and fibroblast in the prepicium of the circumcises patients. The foreskins of the hypospadias group also showed normal epidermis. However, in the dermis of this group, we detected prominent inflammatory cell infiltration. The inflammatory cells were accumulated under epidermis in 5 cases of the hypospadias group (Figure 3). There were also EGFR expression in the endothelial cells and fibroblasts in the prepicium in the same group (Figure 4). The mean EGFR immunostaining scores of foreskin in groups are presented in Figure 5.

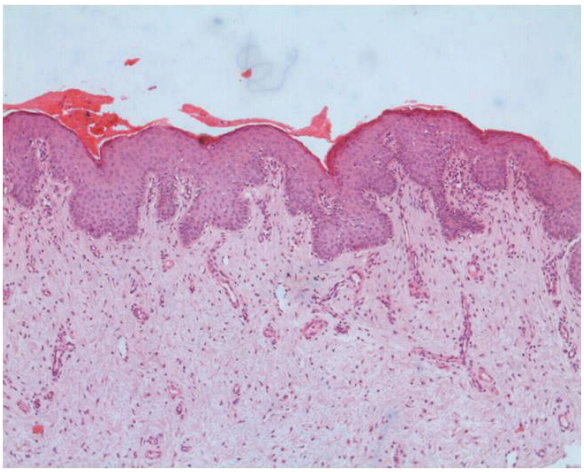

Figure 1. Histology of the foreskin in the circumcision group (H-E, X33).

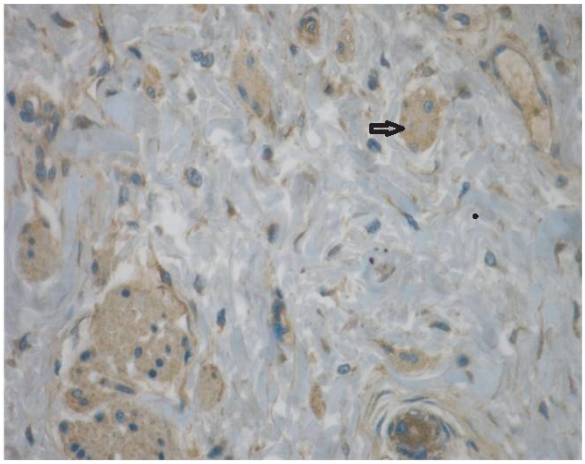

Figure 2. EGFR expression (grade 2) in the smooth muscle, endothelial cells, and fibroblast in the prepicium of a circumcision group patient (x132)

The mean EGFR immunostaining scores in the circumcision and hypospadias groups were 1,57±0,53 and 1,85±0,69 (p=0,42) in the epidermis, 1,71±0,48 and 1,28±0,95 (p=0,53) in smooth muscle, 1,28±0,75 and 1,28±0,75 (p=1) in stroma 1,57±0,78 and 1,28±0,75 (p=0,45) in endothelia of blood vessels, and 1,42±0,78 and 1,28±0,95 (p=0,9) in fibroblasts, respectively. According to these results EGFR immunostaining was not significantly different between the foreskins of the circumcision and hypospadias groups.

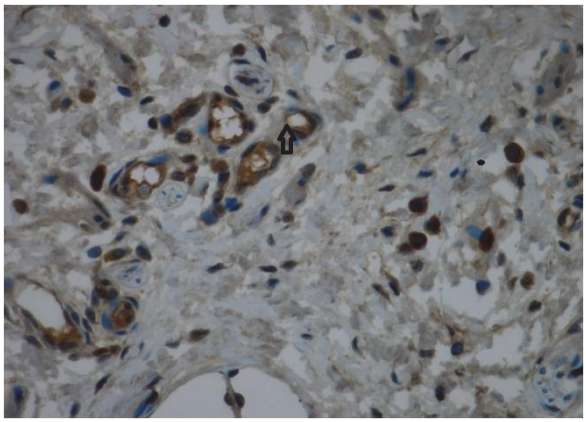

Figure 3. Inflammatory cells under the epidermis in the hypospadias group and the EGFR expression in the epidermis (x66).

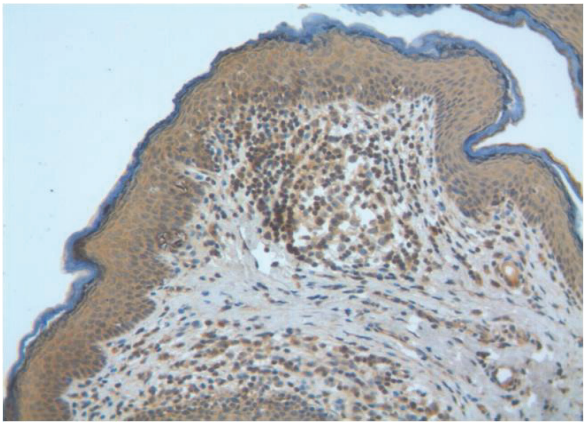

Figure 4. EGFR expression (grade 2) in the endothelial cells, and fibroblasts in the prepicium of a hypospadias patient (x132).

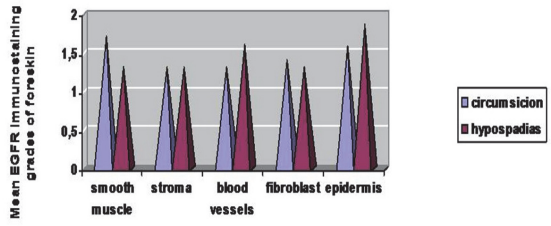

Figure 5. Mean EGFR expression scores ± SD of foreskin in both groups.

The EGFR expression showed increased inflammatory cells in the preputium in children with hipospadias. CD 68 immunostaining for macrophages were negative for these cells. However, toluidin blue staining showed that these inflammatory cells were mast cells. The mean mast cell number in the preputium of children with hypospadias (92±27 / ten fields) was significantly higher than it was in circumcised skin (26±14 / ten fields) (p<0,001) (Figures 6).

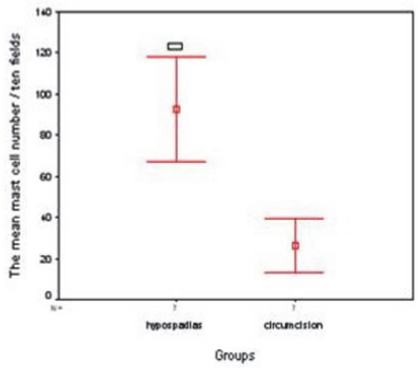

Figure 6. Mean tissue mast cells number / ten fields. Data expressed as mean ± standard deviation (SD). Bar P<0,05 is significantly higher than it is in the hypospadias group.

DISCUSSION

Hypospadias is characterized by incomplete development of the urethra resulting in the external urethral opening getting over the ventral surface of the penis or on the scrotum. Preputial tissue is absent on the ventrum while it is excessive on the dorsal side. It is an anomaly with multiple etiologies (15). A clinical study showed that 64% of children with hypospadias had unknown etiology (3). Recently, an experimental study showed that decreased concentration of EGF in the mice phallus may be associated with the pathogeny of hypospadias. A proper dose of exogenous hCG may increase the concentration of EGF in the mice phalli (16). The differentiation of the reproductive tract into the male phenotype depends on the presence of functional testes, secreting testesteron. The masculinization of the genitourinary tract may be mediated by EGF (17). EGF increases androgen receptor activity via phosphorylation and ubiquitinylation of a cofactor of androgen receptor, melanoma antigen gene protein 11 (18). Another study has shown that inadequate expression of EGF may be related to the etiology of hypospadias (19). In this study, El- Galley et al. report EGFR expression only in the epidermis. However, in our study, we evaluated EGFR expression in both the epidermis and dermis. El-Galley et al. further report inadequate expression of EGF in the periurethral skin as well. But in our study, we investigated EGFR expression in the dorsal foreskin of the hypospadias patients. In our study, we did not find any significant differences in EGFR expression in the preputium of hypospadias patients versus the

circumcision tissue. Hypospadias is a formation defect in the ventral aspect of the penis. According to El-Galley et al., ventral skin exhibited reduces EGFR expression (19).

We showed that the dorsal foreskin has a normal EGFR expression. It is possible that the ventral face could exhibit a reduced expression of EGFR while the dorsal foreskin still has a normal EGFR expression. This issue needs further investigation. Interestingly, we showed that the number of mast cells are increased in the foreskin of our hypospadias patients. Mast cells can produce, store, and release many kinds of chemical mediators, including histamine, tryptase, chymase, heparin proteoglycan, various growth factors, and cytokines. Mast cells can be found in tissues throughout the body, particularly in association with structures such as blood vessels, nerves and in proximity to surfaces that interface with the external environment (20). The induction of new blood vessels, angiogenesis, is related to mast cells. Mast cells accumulate in many angiogenesis-dependent situations, including wound healing, ovulation, and hemangioma (21). The mast cell mediators, such as histamine, vascular EGF, and basic fibroblast growth factors are angiogenic and they regulate endothelial cell proliferation and function (22). Mast cells are known for their roles in allergy, asthma, systemic anaphylaxis, and inflammatory diseases. In tyroid folliculogenesis, thyrocytes and mast cells derived EGF serves follicule regeneration through their mitogenic effects on thyrocytes in an autocrine or paracrine manner (23). Substances produced by mast cells may exert an important effect on embryology, growth, differentiation, and regeneration of intestinal nervous system in the Hirschsprung's disease (24).

Currently, we don't have an explanation as to what might be leading to the observed increase in mast cell numbers in hypospadias patients. An antenatal inflammation could increase the number of mast cells, yet we would expect the numbers to get back to normal once the acute period is over. Considering that the average age of our patients was 30 months, we can rule out a potential inflammation as the reason of increased mast cell numbers in the dorsal preputium of hypospadias patients. A likely speculation is that the increased number of mast cells might serve to secrete more EGF or other substances due to an unknown trigger for reparing ventral preputium defect. Our work has been performed on a small group of patients. This issue needs further investigation with larger group of patients.

CONCLUSION

Throughout our study, EGFR expression was not different between the dorsal foreskins of children with hypospadias and foreskins of children undergoing circumcision. Notably, we have observed an increase in the number of mast cells in the foreskin of the hypospadias patients. Further studies will be necessary to uncover the underlying reasons leading to this observation.

This study has been presented as a poster presentation (Presentation Number: 66) at the 27th National Pediatric Surgery Congress in Malatya between 30 September and 3 October 2009.

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Received/Başvuru: 02.04.2014, Accepted/Kabul: 15.08.2014

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For citing/Atıf için

Gurbuz N, Uguralp S, Ozturk F, Kirimlioglu H. Epidermal growth factor receptor expression in the foreskin of hypospadias patients. J Turgut Ozal Med Cent 2015;22:81-5
DOI: 10.7247/jtomc.2014.2005