FACTORS INFLUENCING POST-CIRCUMCISION WOUND HEALING

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ABSTRACT

Objective: *This study aims to determine the effect of the penile gland and smegma smear, the expression of collagen and fibroblasts in the preputium, on post-circumcision wound healing.* ***Material & Methods:*** *A cross-sectional study conducted in Malang with samples obtained by purposive sampling on September 17, 2022. Inclusive criteria were uncircumcised males who agreed to be circumcised and had no contraindications to the procedure. Furthermore, the preputium obtained from the circumcision procedure was stained with Hematoxylin-Eosin (HE) and counted under an optical microscope. Post-circumcision evaluation was carried out seven days after the procedure. The data were analyzed using the regression method; p denotes the probability value or significance level, while the b score denotes the degree of influence.* ***Results:*** *There were total of 31 research samples that had a significant difference in the average age between the samples with post-circumcision complaints (11.25 ± 2.75) and those without complaints (8.5 ± 2.73) (p=0.05). The regression test found that bacterial culture, fibroblasts, and collagen had significant effects of 24%, 25%, and 24%, respectively.* ***Conclusions:*** *Skin elasticity decreases with age, as marked by increased fibroblasts and decreased collagen density. Fibroblast factors, collagen, and bacterial culture are significant in wound healing.*

Keywords: *Circumcision, collagen, fibroblast, wound healing.*

ABSTRAK

Tujuan: *Mengetahui pengaruh kelenjar penis dan hapusan smegma, ekspresi kolagen dan fibroblas pada preputium, terhadap penyembuhan luka pasca sirkumsisi.* ***Bahan dan Cara:*** *Penelitian cross-sectional yang dilakukan di Malang dengan sampel yang diperoleh secara purposive sampling pada tanggal 17 September 2022. Kriteria inklusi adalah laki-laki yang belum disunat yang setuju untuk disunat dan tidak memiliki kontraindikasi terhadap prosedur ini. Selanjutnya, preputium yang diperoleh dari prosedur sunat diwarnai dengan Hematoxylin-Eosin (HE) dan dihitung di bawah mikroskop optik. Evaluasi pasca sirkumsisi dilakukan tujuh hari setelah prosedur. Data dianalisis dengan menggunakan metode regresi; p menunjukkan nilai probabilitas atau tingkat signifikansi, sedangkan nilai b menunjukkan tingkat pengaruh.* ***Hasil:*** *Terdapat total 31 sampel penelitian yang memiliki perbedaan yang signifikan pada rata-rata usia antara sampel dengan keluhan pasca sirkumsisi (11.25 ± 2.75) dan yang tidak memiliki keluhan (8.5 ± 2.73) (p=0.05). Uji regresi menemukan bahwa kultur bakteri, fibroblas, dan kolagen memiliki pengaruh yang signifikan masing-masing sebesar 24%, 25%, dan 24%.* ***Simpulan:*** *Elastisitas kulit menurun seiring bertambahnya usia, yang ditandai dengan peningkatan fibroblas dan penurunan kepadatan kolagen. Faktor fibroblas, kolagen, dan kultur bakteri sangat penting dalam penyembuhan luka.*

Kata kunci: *Sirkumsisi, kolagen, fibroblast, penyembuhan luka.*

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INTRODUCTION

Circumcision is an operative procedure aimed to remove a part or all of the foreskin or prepuce of the penis.[1-2] This procedure is the most commonly performed in the world. Circumcision aims to prevent the accumulation of smegma in the penis. Smegma is a waxy material secreted by the foreskin glands, along the skin and foreskin mucosa. Smegma accumulating in the foreskin is a good medium for bacteria to multiply. This then results in inflammation and infection. One of the goals of circumcision is to prevent infection and inflammation.[3-4]

Following the circumcision procedure, the skin is injured. It goes through a wound-healing process that includes hemostasis or inflammation, proliferation, and cell remodelling, all of which are regulated by different cells, cytokines, and growth factors.[5] Several pathophysiological and metabolic conditions can cause delayed wound healing, resulting in chronic wounds that persist for more than six weeks. This pathophysiological condition is classified into two types, namely hypertrophic and keloid scar tissue formation.[6] Hypertrophic scarring is associated with a prolonged inflammatory phase increased proteolysis, and disturbances in cell proliferation and migration.[7]

Collagen is the main structural protein in the extracellular matrix in various connective tissues in the body. Collagen plays an important role at every stage of wound healing. Collagen can include homeostasis, interaction with platelets, interaction with fibronectin, increasing fluid exudation, and increasing cellular components. Previous studies have proven that decreased collagen density will interfere with the wound healing process.[6] Fibroblasts are the main elements in the repair process for the formation of structural proteins that play a role in tissue formation. Fibroblasts play a role in degrading fibrin threads and synthesizing extracellular matrix components (collagen, glycoproteins, proteoglycans, and hyaluronic acid).

With age, the production of pro-inflammatory cytokines expressed by collagen and fibroblasts in the prepuce skin will decrease, decreasing function.8 Previous research by Suzana Makpol et al. revealed that collagen expression will decrease with age and the influence of hormones. This study aims to see how the penile gland smear, the smegma smear, and the expression of collagen and fibroblasts in the preputium affect post-circumcision wound healing.

MATERIAL & METHODS

The research design used in this study was cross-sectional, which would determine the effect of age on collagen and fibroblasts in the prostate of post-circumcision patients. Data were taken by purposive sampling on September 17, 2022. The inclusion criteria in this study were uncircumcised men who had normal blood clotting lab results and agreed to have a circumcision. The presence of genital abnormalities, such as the buried penis, micro penis, hypospadias, epispadias, and chordee, was an exclusion criterion.

Preputium skin was taken by circumcision and stored prepuce in 10% formalin. Then paraffin blocks were made and stained with Hematoxylin-Eosin (HE). Collagen and fibroblasts were counted using an optical microscope with 400 times magnification and 10 times field of view.[5] Smegma is taken after the penis is completely retracted. Smegma swabs are carried out using a stick at the base of the wound. After the penis can be completely retracted, a swab is done on the penile gland circularly. Afterward, a culture is done to look for the causative bacteria. Evaluation of surgical wound complications was carried out seven days after circumcision using the Clavien Dindo classification. It is categorized as Clavien Dindo 1 post-circumcision complaint present. Patient complaints in this study included prolonged pain, wound infection, urinary retention, fever, and dysuria. If there are one or more of the complaints, they are included in the group with post-circumcision complaints.

The data obtained were analyzed using the regression method to determine the effect of age on collagen and fibroblasts. The p-value indicates the probability value or significance level, while the b-score indicates the degree of influence.

RESULTS

In the study conducted, it was found that the average age of those who experienced post-circumcision complaints was 11.25±2.75 years; this age was significantly different when compared to patients who did not experience complaints. Likewise, an average BMI experienced complaint of 21.55±1.98. BMI was significantly different when compared to patients who did not experience complaints with a BMI of 19.52±3.81 (Table 1). The presence or absence of smegma also plays a significant role in the presence or absence of complaints. While in penis size, urine pH, and phimosis grade, there were no significant differences between patients who had post-circumcision complaints and those who did not.

The results of the culture of the preputium found that the most bacteria found in the preputium was E. Coli with a total of 13 samples, followed by Enterococcus faecalis with 7 samples, and the least was Staphylococcus epidermidis. Meanwhile, in 9 samples, no bacterial growth was found (Table 2).

The research obtained a sample of 31 participants. The age range was 5 to 14 years. The average number of fibroblast cells/10 visual fields is

15.8-45.6. The average number of all samples is 30.82±7.35. Collagen density obtained between 35.04-51.35, while the average value for collagen is 43.06±4.09 (Figure 1).

It was found an average age of 8.93±2,8. In the regression test, it was found that age had a significant effect on collagen by -67%. This means that with age, the value of collagen density decreases by 67% (Table 3). Meanwhile, as the age of eating fibroblasts will increase by 59%, it can be concluded that the more you age, the more fibroblasts will increase by 59%. These results answer that as age increases, the preputium will be thicker and less elastic.

Collagen, fibroblasts and urine culture were analyzed to determine how influential they were on the wound healing process. In the regression test, it was found that culture had a significant effect of 24% on wound healing. It can be concluded that the more bacteria found, the longer wound healing will be (Table 4). Likewise with fibroblasts, the more fibroblasts, the longer the wound healing process, where fibroblasts have a significant effect of 25%. Collagen has a significant effect on wound healing by 21%, but if the thickness of the fibroblast decreases, the wound healing process will take longer.

Table 1. Research sample description.

Category	No post-circumcision complaints n/(mean)	Post-circumcision complaints n/(Mean)	p-value
Amount (n)	27	4	
Age	8.5 ± 2.73	11.25 ± 2.75	0.04
Body Mass Index (BMI)	19.52 ± 3.81	21.55 ± 1.98	0.05
Penis size	4.77 ± 1.8	5.4 ± 0.84	0.08
Urine pH	6.79 ± 0.45	6.75 ± 0.35	0.7
Phimosis grade:			0.06
1	15	0	
2	4	2	
3	5	0	
4	2	1	
5	1	1	
Smegma			0.05
Yes	21	4	
No	6	0	

Table 2. Culture results of glans penis and smegma smears.

Culture result	N
Sterile	9
E. Coli	13
Enterococcus faecalis	7
Staphylococcus epidermidis	2

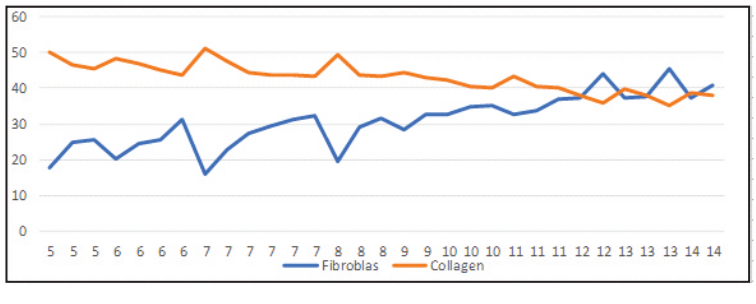

Figure 1. Collagen density value and the number of fibroblast cells against age.

Table 3. Regression test of age with fibroblast and collagen count.

Age	Collagen	Fibroblast	p/(b) collagen	p/(b) fibroblast
8.93 ± 2.8	43.06 ±4.09		0.03/(-0.67)	
		30.8 ± 7.3		0.045/ (0.59)

Table 4. Regression test of penile gland and smegma bacterial culture, collagen and fibroblast towards wound.

Variable	b	p-value
Penile gland and smegma culture smear	0.24	0.05
Collagen	- 0.21	0.05
Fibroblast	0.25	0.04

DISCUSSION

Various factors can hinder the healing process. Non-healing chronic wounds are characterized by specific biological markers. Local and systemic factors contribute to delayed healing. Tissue maceration, foreign bodies, biofilms, hypoxia, ischemia, and wound infection are examples of local factors. Diabetes, old age, malnutrition, and other chronic organ diseases are examples of systemic factors.[6] Even with good clinical practice, it is not possible to eliminate or reduce the impact of these factors. Reduced tissue growth factors, increased proteolytic enzymes like matrix metalloproteinases that break down the extracellular matrix, increased inflammatory mediators like excessive neutrophil infiltration (like in pressure ulcers), and the presence of senescent cells. In addition to local and systemic factors that impair healing, they could be potential biomarkers for chronic wounds.[9]

These changes are largely due to the reduction of type I collagen fibrils, which comprise most of the dermal extracellular matrix (ECM). Prostaglandin E2 (PGE2) is a pleiotropic lipid mediator synthesized from arachidonic acid through the sequential actions of cyclooxygenase (COX) and PGE synthase (PTGES). PGE2 inhibits collagen production by fibroblasts in vitro. Previous studies have shown that PTGES1 and COX2 increase with age in human skin. PTGES1 and COX2 mRNA increased 3.4-fold and 2.7-fold, respectively, in the dermis of elderly people (>80 years) compared to young individuals (21-30 years). In addition, PGE2 levels increase by 70% in the skin with age. Therefore, increasing PGE2 with age will also cause a decrease in collagen production.[10]

In this study, a significant relationship was found between age and the presence of individual complaints after circumcision. The results of this study support the latest systematic review, meta-analysis, and meta-regression studies that circumcision performed under the age of 2 years has the lowest complication rate by a significant margin.[3] Various studies have stated that a low complication rate in young children is associated with better wound healing, including among other things, hypospadias patients who undergo surgery under the age of 6 months have a simpler rate of wound healing.[11] For nearly 50 years, it has been known that fetal wounds heal quickly without scarring.[12] The mechanisms for good wound healing in early childhood are still not fully understood, but the study by Bermudez et al. mentioned that one of the contributing factors is the low production of pro-inflammatory cytokines by genito-urinary fibroblasts and the lack of inflammation.[8] Other factors that can influence are the lifestyles of older children who are more active and have higher mobility, as well as increased sexual activity in adolescents.[3]

Apart from age, the results of the penile gland and smegma smear cultures were also significantly associated with wound healing. This is possible because when the skin is damaged, microorganisms that are normally confined to the surface of the skin gain access to the underlying tissues. The status of infection and replication of microorganisms determines whether a wound is classified as contaminated, colonized, localized infection/critical colonization, and/or disseminated invasive infection. The presence of non-replicating organisms in the wound was defined as contamination, whereas the presence of replicating microorganisms in the wound without tissue damage was defined as colonization. Local infection/critical colonization is a transitional stage that includes the replication of the microorganism and the initiation of local tissue response. The presence of replicating organisms in a wound with subsequent host injury is defined as an invasive infection.[13]

Fibroblasts are the main source of PGE2. Fibroblasts in elderly skin appearance have reduced in distribution due to the fragmentation of collagen

fibrils. Previous studies have shown that reduced distribution/mechanical strength of fibroblasts in elderly skin increases PGE2 production, thereby also contributing to reduced collagen production. In human skin, dermal fibroblasts are responsible for collagen homeostasis.[14] Dermal fibroblasts are embedded in a collagen-rich microenvironment and physically interact with collagen fibrils to maintain normal cell shape and size, which plays an important role in regulating essential cell functions.[14-15]

Collagen is the most abundant protein in mammals, making up about one-third of the whole body's protein content.[16] Collagen is the most important part of the dermis in human skin. It gives the skin its shape and function. During aging, dermal collagen fibrils undergo progressive changes due to age-related fragmentation and disorganization of the collagen fibrils. Alterations in dermal collagen impair the structural integrity of the skin and are associated with age-related disorders, such as increased fragility, impaired blood vessels, and poor wound healing. Moreover, in healthy young skin, dermal fibroblasts interact with intact collagen fibrils; and exert traction force to achieve normal cell shape and size. However, in the old dermis, collagen fibrils are fragmented, which impairs fibroblast-collagen interactions and results in reduced fibroblast distribution, shape, and size.[14,17]

A previous study that performed a urethral examination in patients aged 6 months to 53 years showed that urethral samples had well-vascularized connective tissue.[18] The results of histological and morphometric studies show that there are several age-related structural changes in the foreskin tissue in children aged 0-7 years: arterioles with a lumen diameter of ≥ 0.15 mm are found more in children aged <3 years than those in the age group 3 to 7 years, so the possible risk of postoperative complications of hypospadias is minimal until the age of 3 years because the preputial blood supply is sufficient and inefficient for fibrosis.[5] In addition, an important indicator in postoperative recovery is the ability of the foreskin to contract. A study conducted in England showed that performing surgery at a younger age can make the soft tissues contract, because at a younger age the sensitivity of the prepuce in boys is much lower, while the vascular tissue is less developed. This can prevent massive blood loss and reduce the need to restore nerve fibres.[19]

This study is intriguing as it addresses a relatively understudied area, specifically elucidating the role of fibroblasts, collagen, and bacterial culture in wound healing following circumcision. Additionally, the study delves into the impact of age on post-circumcision complaints, providing valuable insights into the healing process in different age groups. The inclusion of these detailed analyses sets this study apart from more general studies on circumcision complications and wound healing.

The limitations of this study include a relatively small sample size, inadequate duration of complication follow-up, and the utilization of only dorsumcision technique. These factors may limit the generalizability of the findings. Additionally, the cross-sectional design of the study may not establish causality between the variables examined. Further longitudinal studies with larger sample sizes, use various circumcision technique, and longer duration for follow up are needed to confirm and expand upon the results observed in this research.

CONCLUSION

Skin elasticity decreases with age, as marked by increased fibroblasts and decreased collagen density. Fibroblast factors, collagen, and bacterial culture are significant in wound healing.

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