



The Effect of Amniotic Membrane on the Healing of Cesarean Wounds: A Randomized Clinical Trial

Zahra Molazem,¹ Fatemeh Mohseni,² Mahnaz Rakhshan,^{3,*} Sareh Keshavarzi,⁴ and Masoumeh Younesi⁵

¹Community Based Nursing and Midwifery Research Center, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, IR Iran

²Student at Research Committee, Shiraz University of Medical Sciences, Shiraz, IR Iran

³Medical-Surgical Nursing Department, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, IR Iran

⁴Department of Bio Statistics, Shiraz University of Medical Sciences, Shiraz, IR Iran

⁵Gerash Amir-al-Momenin Medical and Educational Center, Gerash, Fars, IR Iran

*Corresponding author: Mahnaz Rakhshan, Medical-Surgical Nursing Department, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Zand Blv, Namazi Square, Shiraz, IR Iran. Tel: +98-7136474254, E-mail: mzhakhshan@gmail.com

Received 2017 April 16; Revised 2017 June 20; Accepted 2017 August 16.

Abstract

Background: Along with an increase in the rate of cesarean sections, the complications and problems associated with this procedure have also increased in Iran. Factors such as complications associated with caesarean scars, the high cost of chemical treatments, and failure of medications in treatments have led to an increased use of traditional and biological therapies in the healing and preventing of cesarean wound infections.

Objective: To determine the effect of an amniotic membrane dressing on cesarean wound healing.

Methods: This study is a prospective, randomized double-blind clinical trial. Patients participating in the study were women who underwent cesarean sections at Amir-al-Momenin Hospital in Gerash, Iran. Patients were randomly divided into two groups (N = 45 for each group). In one group, the cesarean wound was dressed using an amniotic membrane; in the control group, the dressing was performed using a simple dressing. The wound healing was assessed by the Redness, Edema, Ecchymosis, Discharge, Approximation (REEDA) scale 24 hours and 8 days after the cesarean procedure.

Results: The average REEDA score, reflecting wound healing was significantly different between the groups 24 hours after the cesarean section, which proved to be less in the amniotic membrane group ($.00 \pm .00$ vs. 0.60 ± 1.30 ; $P = .003$). However, on the 8th day after the cesarean section, there was no significant difference between the groups ($P = 0.078$).

Conclusion: The findings of this study showed that the application of an amniotic membrane dressing can be helpful in early stage wound healing of cesarean.

Keywords: Amnion, Cesarean Section, Wound Healing

1. Background

The World Health Organization (WHO) proposed the maximum rate of the cesarean procedure to be 15% based on actual and practical indications (1). Regardless of this statistic, the number of cesarean sections performed varies in different parts of the world and is constantly increasing (2). The rate of cesarean sections in Iran is estimated to be about 40 to 50 percent. Along with an increase in cesarean rates, the complications and problems associated with the procedure have also increased. The increased rate of cesarean sections in Iran has resulted in health concerns for many experts and policymakers (3).

These complications include infection, wound dehiscence, necrosis of the fascia, peritonitis, and scarring that can lead to a longer stay in the hospital. In addition, in-

terventions such as wound drainage, debridement, and repair may be required, all of which impose a significant economic burden on families. Some researchers believe that postpartum economic and mental burdens imposed on the mother and society can lead to more difficult for the mother and baby to develop a psychoemotional bond (4, 5).

Moreover, due to further complications such as pain and morbidity associated with cesarean section, it can also cause a delayed start of breastfeeding and neonatal weight loss in the early days following birth (6). Proper wound care after surgery should produce effective wound healing. Factors such as complications, the high cost of chemical treatments, and failure of medications in treatments has led to an increased use of traditional and biologic therapies in the healing and preventing of cesarean wound in-

fections.

One of the proposed methods of wound care is the use of amniotic membrane. Amniotic membrane was used in 1910 to cover wounds. This method was used for the treatment of third degree burns, resulting in positive results (7). The use of amniotic membrane to treat corneal ulcers in rats caused an increase in corneal re-epithelialization and accelerated wound healing (8). Additionally, it was also effective in the treatment of diabetic foot ulcers (9). Previous studies have shown that amniotic membrane has the following effects: it increases proliferation (10), promotes the development, enhancement, and progression of the healing process (11), demonstrates a lack of immunological effects (12), decreases inflammation (13), decreases scar tissue formation (14), develops antibacterial agents (15, 16), decreases pain at the dressing site (17, 18), creates a natural biological barrier (12, 18), and contains a number of essential factors for growth and cytokines (19). Therefore, amniotic membrane dressing is suitable for all types of wounds. This method is a cost effective way of dressing cesarean wounds. If amniotic membrane is used on the wound of a patient, complications such as transmission of infectious diseases (e.g., HIV, hepatitis) are also minimized (20).

However, there is still uncertainty about the effect of amniotic membrane on surgical wounds. In Iran, controlled trials examining the effect of amniotic membrane on cesarean wound healing is limited and there are no studies exploring the effects of fresh amniotic membrane on cesarean wound healing. Given the high rate of cesarean sections performed in Iran and the associated complications, we decided to do a study on the effects of fresh amniotic membrane on the wound healing of cesarean section.

2. Methods

2.1. Study Design

This study is a prospective, randomized double-blind clinical trial in which the effect of amniotic membrane on wound healing of cesarean section was compared with a control group at the Amir-Al-Momenin Hospital in Gerash, Iran.

2.2. Setting and Sample

Before the study, written permission of the Ethics Committee of Shiraz University of Medical Sciences (code: 93 - 6993) and a registration code from the Iranian Registry of Clinical Trials (IRCT) (code: 2014010616110n1) were obtained. According to similar studies (21, 22) and researcher's knowledge, the sample size for this study was calculated approximately as 45 patients in each group

based on an alpha level of 0.05, SD of 0.4, mean difference of 0.24 and a power of 80%. Considering the probable patient dropout rate, the number of patients in each group was increased to 50. Participants in this study were mothers aged 17-35 years and with gestational ages of 37 - 42 weeks. Enrolled mothers were randomly assigned into control group (n=49) and intervention group (n=50) with the use of randomization list (random permuted blocks with length 4). The purpose of the study, potential harms, benefits, and the nature and duration of the study were explained to the patients and they were reassured that their information would remain confidential. The participants were also informed of their right to discontinue participation, if desired, at any time. Patients participating in this study were visited by a researcher at the hospital before surgery. The inclusion criteria included: 1) term pregnancy (42 - 37 weeks), 2) body mass index (BMI) greater than 29, 3) willingness to participate in the study, 4) no complications of pregnancy (e.g., eclampsia, placenta previa, placental abruption, chorioamnionitis, polyhydramnios), 5) no history of any known disease that would affect wound healing, 6) absence of fetal abnormalities and infants not having been transferred to the neonatal intensive care unit (NICU), 7) no major bleeding or need for blood transfusion, 8) no history of myomectomy, 9) no history of smoking cigarettes, alcohol or illicit drug use, 10) no history of preoperative rupture of membrane, 11) absence of meconium defecation, and 12) no history of cesarean section more than twice. Women were excluded from the study if the operation was prolonged for more than 90 minutes, or if the patient did not refer to the hospital 8 days after surgery.

2.3. Measurements / Instruments

To collect data from the patients, we used a demographic questionnaire and the REEDA scale to ensure that reliability and validity were verified (23). In similar studies, this scale has also been used (21, 24).

Using a demographic questionnaire, data from mothers regarding their age, occupation, gestational age, parity, neonate sex, duration of surgery, duration of preoperative nothing by mouth (NPO) status, height, weight, BMI, blood pressure, heart rate, temperature, and type of anesthesia were obtained. Healing status was assessed using the REEDA scale. The REEDA scale is a tool for measuring the healing process of an incision related to childbirth with acceptable psychometric properties (21, 24). It has five components including redness, edema, ecchymosis, discharge, and approximation of the two edges of the wound. In this scale, a score ranging from 0-3 was awarded to each component. A score of "0" means there is no sign of a particular component, while "3" is the highest score, indicating the presence of one of the five signs.

The scores obtained for each component were summed up and a total score ranging from 0 - 15 was recorded. Higher final scores indicated poor wound healing. For 20 of the participants, wound healing was determined one time by a researcher and at another time by an expert consultant using the REEDA scale. Subsequently, the findings were compared and the coefficient correlation between the data was calculated ($r = 0.79$).

2.4. Intervention

The researcher and the patients were unaware of the type of intervention. This was achieved because the application of the amniotic membrane dressing was performed by a surgeon and the evaluation was done by a researcher. After receiving written consent and collecting demographic data, the participating patients were introduced to the gynecologist. A list of random numbers (prepared by the researcher) generated by permuted block randomization was given to the gynecologist so that the gynecologist could divide patients accordingly into two groups the amniotic membrane dressing group and the control group. All of the mothers who had been studied received 2 g of cefazoline, 50 mg of ranitidine, and 10 mg of metoclopramide injected intravenously in the morning, before surgery; all of them received the same pre-surgery preparation. The mothers' preoperative preparation included routine steps that typify surgery in general, and the skin of the surgical area was scrubbed with betadine and then draped. Women in this study underwent cesarean section involving the Pfannenstiel skin incision and the low transverse uterine cesarean incision, performed by the same surgical gynecologist. In all cases, the skin was sutured using plastic nylon thread No. 30. In the experimental group, amniotic membrane was used. In this procedure, the gynecologist separated the amniotic membrane from the placenta using sterile gloves. After washing it with normal saline and cleansing the membrane from blood and clots, the amniotic membrane was placed on the surgical wound of the patients in the experimental group. To prevent rapid drying of membrane, wet sterile gauze saturated with saline was placed on it and then the wound was dressed with a few pieces of dry gauze. In the control group, the dressing was done with dry gauze alone. Then, 24 hours after surgery, wound healing was assessed by a researcher using the REEDA scale, after removal of the dressing by nurses. In this study, checking of the wound and scoring was carried out by the researcher and the intervention was performed by the surgical gynecologist. Furthermore, the wound was cleaned and amniotic membrane completely covered the wounds.

All patients stayed one night in the hospital and received 4 g of intravenous Keflin in 4 divided doses, every

6 hours. Subsequently, cephalixin capsules (500 mg) were administered, 4 times a day for 7 days. After they were transferred to the surgery ward, a diclofenac suppository was administered to them. Mefenamic acid (250 mg) was given orally every 8 hours during the first three days.

In all cases, the neonates were breast-fed immediately after birth and after the mothers were transferred to the maternity ward. In all cases, the uterine was contracting normally and vaginal bleeding was normal in the recovery ward. Bleeding from surgical sites was not found.

Infection symptoms such as redness, discharge, edema, etc. were taught to patients. All patients were educated about hygiene and nutritional needs. At the time of discharge from the hospital, the next visit was determined to be 8 days after surgery. The study groups were observed via telephone and if they reported symptoms of infection, they visited a specialist and were treated. On the appointed day, wound healing was assessed using the REEDA scale and also recorded. Subsequently, the sutures were removed.

2.5. Data Analysis

In this study, data were analyzed by SPSS software (IBM, Armonk, NY, USA, version 19). For analysis of data, the chi-square test and t-test were used.

3. Results

After the screening phase between 4 May and 5 July 2014, 145 women were assessed for eligibility. Of these women, 99 met the selection criteria and were randomized to the amniotic membrane or control group. At the end of the study, 9 patients were lost to follow up. Forty-five women were deemed eligible for analysis in the amniotic membrane group, while 45 women were assigned to the control group (Table 1).

The average of variables of the two groups were compared. The two groups were homogeneous for age, education, gestational age, parity, history of abortion, neonate sex, method of anesthesia, duration of NPO period, duration of surgery, height, weight, heart rate, systolic and diastolic blood pressures, and body temperature before surgery ($P > 0.05$). Regarding BMI ($P = 0.012$) and postoperative temperature ($P = 0.007$), there was a significant difference between the groups.

Healing scores using the REEDA scale in the 2 groups were evaluated for 24 hours postoperatively and 8 days after surgery. The results were then compared. The score of cesarean wound healing based on the REEDA scale 24 hours postpartum was (0.00 ± 0.00) in the amniotic membrane group and (0.60 ± 1.30) in the control group. This difference was statistically significant ($P = 0.003$). On the eighth

Table 1. Comparison of Demographic Variables Between the Two Groups^a

Characteristic	Amniotic Membrane Group (n = 45)	Control Group (n = 45)	P-Value
Age, years	27.38 ± 3.92	26.62 ± 4.88	0.420
Gestational age, week	38.56 ± 1.10	38.73 ± 1.12	0.448
NPO time, h	7.93 ± 1.39	7.96 ± 1.92	0.950
Operation time, min	39.40 ± 4.33	39.44 ± 4.47	0.962
BMI, kg/m ²	30.12 ± 1.16	30.80 ± 1.35	0.012
Systolic blood pressure immediately after operation, mm Hg	114 ± 11.86	117 ± 11.45	0.323
Diastolic blood pressure immediately after operation, mm Hg	75.44 ± 7.00	77.69 ± 6.22	0.111
HR	78.42 ± 10.01	76.78 ± 11.51	0.471
Temperature before operation, Celsius	37.02 ± .26	37.05 ± .25	0.544
Temperature immediately after operation, Celsius	36.90 ± .22	37.08 ± .38	0.007

Abbreviations: NPO, not per oral; BMI, body mass index; HR, heart rate.
^aValues are given as mean ± SD.

Table 2. Comparison of the Frequency of Demographic Variables Between the Two Groups^a

	Amniotic Membrane (%) (n = 45)	Control (%) (n = 45)	P-Value
Education, diploma and more	31 (68.9)	29 (64.4)	0.815
Primary gravid	20 (44.4)	24 (53.3)	0.385
Abortion history	7 (15.6)	6 (13.3)	0.841
General anesthesia	36 (80)	35 (77.8)	0.796
Neonatal sex, male	22 (48.9)	27 (60)	0.290

^aValues are given as number (percentage)

day postpartum, the score of the REEDA scale in the amniotic membrane group was (0.02 ± 0.15) and (0.29 ± 0.99) in the control group; in fact, the score of the REEDA scale in the amniotic membrane group was less than that in the control group. Although these differences were not significant (P = 0.078).

Table 3. Comparison of the Score of Wound Healing Between the Two Groups^a

Group	Amniotic Membrane Mean (n = 45)	Control Mean (n = 45)	P-Value
REEDA scale, 24 h postpartum	0.00 ± 0.00	0.60 ± 1.30	0.003
REEDA scale, 8th day postpartum	0.02 ± 0.15	0.29 ± 0.99	0.078

Abbreviations: REEDA, Redness, Edema, Ecchymosis, Discharge, Approximation.

^aValues are given as mean ± SD.

The number of participants in the amniotic group who had a REEDA score of "0" 24 hours and 8 days after surgery was more numerous than that of the control group. This demonstrates better healing 24 hours after surgery in the amniotic membrane group (Table 3).

None of the patients of the amniotic membrane group had complications related to wound infection. Due to a lack of complete healing and symptoms of infection, about 7% of patients (3 patients) in the control group were prescribed additional doses and types of antibiotics (cefixime, 400 mg orally, once a day for 10 days, and gentamicin, 60 mg IM, once a day for 5 days). Their stitches were withdrawn a few days later, after complete healing.

4. Discussion

Many previous studies examining the effects of amniotic membrane on wound healing confirm the results obtained in the present study.

We studied its effect on healing cesarean wounds for the first time. Previous studies used sterilized and frozen, dried amniotic membrane (7, 9, 25). Presumably, some of the material and cells were lost in these processes. Use of fresh amniotic membrane is one of the benefits presented in this research. Amniotic membrane consists of three main types of material, including building collagen, extracellular matrix, and biologically active cells and molecules that are important in the healing process of a wound (26). The REEDA scores observed between the amniotic membrane and control groups were significant at 24 hours postpartum, but on the eighth day, postpartum they did not show significant differences. However, overall, wound healing was better in the amniotic membrane group than in the control group. The the application of the amniotic membrane during the 24 hour postoperative period, which could explain the obtained results. In a study conducted on 53 rats, Miettinen et al. found similar results (27). His findings showed that the amniotic membrane is

effective during the first phase of healing, but in the second phase of healing, it has no significant impact.

In a study with a small sample size (4 patients), Alsina and Pedergosa examined the effect of the amniotic membrane on chronic wounds of the distal extremity and reported positive effects that are consistent with our study (25). Zelen et al. also used the amniotic membrane on diabetic foot ulcers and reported that it was effective (9). In a clinical trial study, Mohammadi et al. used amniotic membrane on second and third-degree burn wounds that required skin grafts, and the skin grafts were covered with amniotic membrane. The results showed that use of the amniotic membrane significantly reduces the duration of complete graft take (7). Our study showed that amniotic membrane can be helpful in early stage wound healing of cesarean.

4.1. Limitations

Limitations of this study are those inherent to small sample size. Our findings should be confirmed and expanded with subsequent clinical trials. Other one of the limitations of this study was the lack of careful assessment of the wound healing status via biopsy of the wound. In addition, an inability to control nutrition status in the home, overall health status, and the level of physical activity of each person, as well as the effect of these factors on wound healing are limitations of this study. However, there was an effort made to control this problem using similar patient training and random sampling.

4.2. Conclusions

This study showed that the amniotic membrane is effective in the early phase of wounds healing of cesarean section and can be used in addition to conventional therapies. Based on our findings, the amniotic membrane can be used as a dressing aid to assist healing after a cesarean section, without the use of excessive costs and complex interventions as strategies to achieve maternal health. In addition, conducting a similar study with a larger sample size is recommended.

Acknowledgments

This study was approved by the Institutional Review Board of the Shiraz University of Medical Sciences (IRB approval number: 93-6993) and registered in Iran Registry of Clinical Trials (IRCT No. IRCT 2014010616110n1) of the Ministry of Health and Medical Education.

Footnotes

Conflict of Interest: The authors have no conflicts of interest.

Funding Support: This study was approved and financially supported by the Institutional Review Board of the Shiraz University of Medical Sciences, Shiraz, Iran.

References

- Moore B. Appropriate technology for birth. *Lancet*. 1985;**326**(8458):787.
- Danforth DN, Gibbs RS. *Danforth's obstetrics and gynecology*. Lippincott Williams and Wilkins; 2008.
- Azami-Aghdash S, Ghojzadeh M, Dehdilani N, Mohammadi M, Asl Amin Abad R. Prevalence and Causes of Cesarean Section in Iran: Systematic Review and Meta-Analysis. *Iran J Public Health*. 2014;**43**(5):545-55. [PubMed: 26060756].
- Samadi S, Khadivzadeh T, Emami A, Moosavi NS, Tafaghodi M, Behnam HR. The effect of Hypericum perforatum on the wound healing and scar of cesarean. *J Altern Complement Med*. 2010;**16**(1):113-7. doi: 10.1089/acm.2009.0317. [PubMed: 20064022].
- Sarsam SE, Elliott JP, Lam GK. Management of wound complications from cesarean delivery. *Obstet Gynecol Surv*. 2005;**60**(7):462-73. [PubMed: 15995563].
- Dahifar H. Early weight loss of breast-fed neonates born under C-section in their first 48 hours of life. *Para Med Sci*. 2003;**1**(4):203-10.
- Mohammadi AA, Johari HG, Eskandari S. Effect of amniotic membrane on graft take in extremity burns. *Burns*. 2013;**39**(6):1137-41. doi: 10.1016/j.burns.2013.01.017. [PubMed: 23523222].
- Yeh LK, Chen WL, Li W, Espana EM, Ouyang J, Kawakita T, et al. Soluble lumican glycoprotein purified from human amniotic membrane promotes corneal epithelial wound healing. *Invest Ophthalmol Vis Sci*. 2005;**46**(2):479-86. doi: 10.1167/jovs.04-1014. [PubMed: 15671272].
- Zelen CM, Serena TE, Denozziere G, Fetterolf DE. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. *Int Wound J*. 2013;**10**(5):502-7. doi: 10.1111/iwj.12097. [PubMed: 23742102].
- Cornwell KG, Landsman A, James KS. Extracellular matrix biomaterials for soft tissue repair. *Clin Podiatr Med Surg*. 2009;**26**(4):507-23. doi: 10.1016/j.cpm.2009.08.001. [PubMed: 19778685].
- Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. *Exp Eye Res*. 2000;**70**(3):329-37. doi: 10.1006/exer.1999.0794. [PubMed: 10712819].
- Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cells Mater*. 2008;**15**:88-99.
- Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea*. 2000;**19**(3):348-52. [PubMed: 10832697].
- Lee SB, Li DQ, Tan DT, Meller D, Tseng SC. Suppression of TGF- β signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. *Curr Eye Res*. 2000;**20**(4):325-34.
- Sangwan VS, Basu S. Antimicrobial properties of amniotic membrane. *Br J Ophthalmol*. 2011;**95**(1):1-2. doi: 10.1136/bjo.2010.184259. [PubMed: 21163818].
- King AE, Paltoo A, Kelly RW, Sallenave JM, Bocking AD, Challis JR. Expression of natural antimicrobials by human placenta and fetal membranes. *Placenta*. 2007;**28**(2-3):161-9. doi: 10.1016/j.placenta.2006.01.006. [PubMed: 1653165].
- Adly OA, Moghazy AM, Abbas AH, Ellabban AM, Ali OS, Mohamed BA. Assessment of amniotic and polyurethane membrane dressings in the treatment of burns. *Burns*. 2010;**36**(5):703-10. doi: 10.1016/j.burns.2009.09.003. [PubMed: 20004061].

18. Mermet I, Pottier N, Sainthillier JM, Malugani C, Cairey-Remonnay S, Maddens S, et al. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. *Wound Repair Regen.* 2007;**15**(4):459-64. doi: [10.1111/j.1524-475X.2007.00252.x](https://doi.org/10.1111/j.1524-475X.2007.00252.x). [PubMed: [17650088](https://pubmed.ncbi.nlm.nih.gov/17650088/)].
19. Parolini O, Solomon A, Evangelista M, Soncini M. Human term placenta as a therapeutic agent: from the first clinical applications to future perspectives. Human placenta: structure and development. *Nova Sci Pub.* 2010:1-48.
20. Mamede AC, Botelho MF. *Amniotic Membrane: Origin, Characterization and Medical Applications*. Springer; 2015.
21. Heidari T, Roozbahani N, Farahani LA, Attarha M, Torkestani NA, Jamilian M. Does Iranian *Astragalus gossypinus* honey assist in healing caesarean wounds and scars?. *EujlM.* 2013;**5**(3):226-33.
22. Razmjoo N, Adeli M, Tara F, Ebrahimzadeh S. The effect of early post cesarean feeding on wound healing. *IJN.* 2012;**3**:15-6.
23. Hill PD. Psychometric properties of the REEDA. *J Nurs Midwif.* 1990;**35**(3):162-5.
24. Razmjoo N, Adeli M, Tara F, Ebrahimzadeh S. Effect of Early Oost Cesarean Feeding on Wound Healing. *Horizon Med Sci.* 2013;**19**(3):83-8.
25. Alsina-Gibert M, Pedregosa-Fauste S. Amniotic membrane transplantation in the treatment of chronic lower limb ulcers. *Actas Dermosifiliogr.* 2012;**103**(7):608-13. doi: [10.1016/j.ad.2012.01.010](https://doi.org/10.1016/j.ad.2012.01.010). [PubMed: [22578292](https://pubmed.ncbi.nlm.nih.gov/22578292/)].
26. Schmidt W. The amniotic fluid compartment: the fetal habitat. *Advanc Anatom Embryol Cell Biol.* 1991;**127**:1-100.
27. Nordback PH, Miettinen S, Kaariainen M, Pelto-Huikko M, Kuokkanen H, Suuronen R. Amniotic membrane reduces wound size in early stages of the healing process. *J Wound Care.* 2012;**21**(4):190-196-7. doi: [10.12968/jowc.2012.21.4.190](https://doi.org/10.12968/jowc.2012.21.4.190). [PubMed: [22584678](https://pubmed.ncbi.nlm.nih.gov/22584678/)].