Efficacy Evaluation of Amniotic Membrane in the Treatment of Neonatal Extravasation: a Pilot Study

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Research Article

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Abstract

Intravenous treatment exposes the neonates to extravasation due to fragile and small veins and the long period required for treatment. Extravasation is leakage of fluids, nutrition, or drugs from a peripheral intravenous which could cause tissue damage. The injured complications range from local irritation to skin necrosis and severe scar formation after the healing. Several methods have been used to control the complications of extravasation. We used an Amniotic membrane, a biological dressing, for healing the wounds. Our object in this study is to examine whether the amniotic membrane can induce healing wounds following extravasations.

This prospective 13-week single-arm clinical trial study was performed on five neonates from February 2020 till May 2021 in the children's medical center of Tehran University. Neonates with any gestational age and diagnosis of the wound due to extravasation entered our study. Neonates with skin disorders and wound stages of 1 and 2 were excluded from the study. Established wounds without necrosis and infection are treated with an amniotic membrane. The amniotic membrane covers the wound, and after 48 hours, the wound is rechecked. The sequence of replacing or removing the bandages is five to seven days until healing occurs.

An amniotic membrane was applied to the wounds and the average time for healing was 2.5 weeks. The average gestational age was 33.6 weeks. We did not report any adverse reaction, and healing was without scar formation.

Implementing an amniotic membrane for treating wounds due to Extravasation can be a new approach. This treatment route decreases graft requirement and can be implemented by expert nurses, so in remote NICUs, its usage is easy.

Summary:

What is Known:

- Amnion membrane has shown promising effects in treatment of chronic and acute wounds, Epithelial defects, eye disorders, diabetic foot ulcer and other related indications.[1]
- Amniotic membrane has five layers containing anti-angiogenic, anti-scacring, anti-fibrotic, anti-microbial and anti-inflammatory properties resulting in healing wounds more quickly[2].

What is New:

- AM can be a new and hopeful method to heal wounds due to extravasation more quickly as it has shown promising effects in chronic and acute wound re-epithelization.
- Tissue engineering requires native biomaterials such as AMT that has extracellular matrix containing growth factors and cytokines to avoid invasion by fibrotic tissue.

Introduction:

Sick or premature neonates are exposed to many medications. Drug absorption via the gastrointestinal tract is influenced by slow gastric emptying and intestinal motility. Gastrointestinal flora influences drug absorption. Gut flora varies due to gestational age, delivery route, age, and feeding [3]. Additional factors such as enzymes metabolizing drugs, gut transporters are not entirely developed [4]. Due to these factors, oral medications are ineffective, so intravenous medication is the mainstay treatment in neonates. Intravenous treatment exposes the neonates to Extravasation due to fragile and small veins and the long period required for treatment [5]. Extravasation is leakage of fluids, nutrition, or drugs from a peripheral intravenous which could cause tissue damage [6]. Based on extravasated material, volume, and patient-related factors, the injured complications range from local irritation to skin necrosis and severe scar formation after the healing [7]. Several methods have been used to control the complications of Extravasation. The best management is preventative approaches and early treatment. Based on the severity of the injury, extravasations are classified into four stages. Most extravasation injuries are Grades 1 & 2 and do not require treatment [8]. Many interventions are introduced for treatment as hyaluronidase [9], saline irrigation [10], Topical nitroglycerin 2% ointment [11], phentolamine [12], Subcutaneous Terbutaline [13]. Another method that has been noted recently is the usage of the Amniotic membrane (AM). AM, a biological dressing, has been used for various types of wounds [14]. This product was widely used in ophthalmology by success due to its anti-inflammatory properties [15]. Yanxia Hao and et al. postulated that the human amniotic membrane contains many anti-inflammatory and antiangiogenic proteins like IL-1ra, IL-10, and collagen a1(XVIII) (Col XVIII, the precursor protein of endostatin; thrombospondin-1 (TSP-1); the tissue inhibitors of metalloproteinase (TIMP-1, -2, -3, -4) [16]. AM, also known as AM allograft, has several benefits on wounds due to the extracellular matrix that contains several growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), some other cytokines including TNF-α, IL-1, IL-10 as well as viable cells [17]. AM was reported to be effective in wound healing with the lowest scar preformation, decreasing in time of re-epithelization and infection. It has been decades that the AM uses for the treatment of acute and chronic wounds in the following cases: ocular amniotic membrane transplantation (AMT) and surgery, burn wounds, diabetic foot ulcers, surgical incisions [18]. To date, there are few reports of the implementation of this product in the pediatrics field. AM was implemented successfully in pediatric burn injuries and ocular complications due to Stevens-Johnson disease [19, 20]. The potential to induce proliferation and wound healing without hazardous complications conceived us to try this product in neonatal extravasations. Our object in this study is to examine whether can the amniotic membrane induce wound healing following extravasations.

Material And Methods:

Study design and population:

This nonrandomized single-arm clinical trial study was performed within 13 weeks on four neonates from February 2020 till May 2021 in the children's medical center of Tehran University. Our NICU is a tertiary

referral center for caring for transformed neonates from the whole of Iran due to its facilities.

Inclusion criteria:

Neonates from the first day of life and infants till three years of age with extravasation entered the study.

Exclusion criteria:

Neonates with congenital skin disorders and wound stages of 1 and 2 were excluded from the study.

Study Methods:

The standard care following extravasation was performed to minimize the injury and prevent the wounds with stage 2 or higher, even though it leads to enrollment prolongation. The infusion is stopped, and the extremity is elevated. A saline-soaked gauze is placed on the injury to draw out the vesicant [21]. 48–72 hours after extravasation was established[22], 20 multidiscipline experts examined the wound for treatment. The implemented AM is a product of (Sinacell-Iran). Wounds containing necrosis were debrided by autolytic debridement. The implemented autolytic is a hydro clean plus (Hartmann).

In cases with slough, a culture was sent to the laboratory. Empirical intravenous antibiotics with topical mupirocin were started till the infection resolved. After the necrotic tissue was removed and the slough was resolved, AM was implemented on the wound since AM should be placed on wounds without necrosis and slough. The Vaseline gauze was placed as the secondary dressing on AM. The wound was evaluated visually after 48 hours through the transparent AM for any unwanted events. Then, the AM was replaced with a new sheet AM five days after the first bandage. This procedure was repeated till the wound epithelization. Before each dressing, we took a photo in order to compare the progression of healing.

Adverse Events (AEs):

The AEs and their severity were recorded according to the Common Terminology Criteria v 5.0 for AEs (CTCAE) [23]. AEs were documented within the first month after the intervention.

Primary outcome:

The primary outcome was time to epithelization.

Secondary outcomes:

The secondary outcome was scar forming after wound treatment, hospitalization period, and occurrence of sepsis.

Sample size:

It was assumed that the epithelization time for extravasation stages III & IV without AM would be 60 days on average, and the intervention with the AM would reduce the time to 15 days [24]. A sample size of 4 patients achieves 100% power to detect a difference of 45 days between the hypothesis mean of 60 days and the alternative hypothesis mean of 15 days with an estimated standard deviation of 5.0 and with a

significance level (alpha) of 0.05 using a two-sided Wilcoxon test assuming that the actual distribution is normal. Pass version 11 was used to sample size calculation.

Results:

A public call was given to children's hospitals in order to refer extravasations for treatment. Seven patients have enrolled in the study. Three of them were excluded from the study. One patient did not meet the criteria, and the other was an infant and did not consent. The third patient's living place was remote, and they could not attend our hospital. After four weeks of follow-up, no adverse reaction was reported and healing was without a scar.

Table 1
Demographic characteristics

Case Number	Sex	GA (week)	Birth Weight (g)
Case 1	F	29	1200
Case 2	M	30	1350
Case 3	M	37	2670
Case4	M	38	3800

Table 2
Wound characteristics

Case Number	Site of Injury	Wound Grading	Extravasated Liquid	Injury extent (cm)	Debridement Need	Number of membranes required (n)
Case 1	Right foot	3	Blood	50*40	Yes (Autolytic)	4
Case 2	Left foot	3	DW12.5%	20*25	Yes	2
Case 3	Left foot	3	DW10%	10*20	No	2
Case 4	Left foot	3	Dw12.5%	20*25	Yes (Autolytic)	2

Discussion:

Our study showed an average of 2.5 weeks of wound healing following extravasation regardless of the causative agent. The duration of healing seems to be related to the wound extent. We reported no adverse reactions, and scar formation was not seen. The medical staff and nurses were satisfied as the care of the wound was easy. Infection following the dressings were not reported. We implemented the AM in two

premature neonates that the result was acceptable. Extravasations in preterm are due to more extended requirements for intravenous therapy and fragile vessels. The cause in term neonates is more due to the rapid and vigorous limb movements during hunger, agitation, or handling by the mother, that displaces the catheter and tears the vessel.

In both cases, the fluid leaks into the soft tissue. Based on the fluid that escapes the wall, they are divided into infiltrates and vesicant fluid. In infiltration fluids as normal saline, exert mechanical forces on blood supply, lymphatic drainage, and subdermal plexus. The result is occlusion of blood vessels that causes ischemia and finally necrosis [25]. The amount of accumulated fluid shows the extension of injury. Vasoconstriction and ischemia are induced by vasopressors such as dopamine and dobutamine that cause necrosis [26]. Hyperosmolar fluids as hypertonic glucose result in cell shrinking by shifting water from the cell to the interstitial tissue [27]. The injury mechanism of vesicant fluids is both mechanical pressure and cell death. They lead to chemical burning of the skin. Drugs with a PH out of the normal range of blood cause cell damage. Phenytoin and vancomycin are examples of this mechanism [21]. Extravasation of total parenteral fluids, calcium gluconate, can cause necrosis. Tissue injury maybe not be recognized until 48 to 72 hours after the leak. Besides, newborns cannot react to the first stages of injury, so some cases are identified when necrosis is established [28]. There are specific treatments for each mentioned above extravasation mechanism. Hyaluronidase is better administered within the first two hours after extravasation in five separate sites [29].

Phentolamine is the antidote to vasoactive drugs such as dopamine and dobutamine and should be administered within 12 hours after injury. Administration in neonates should be followed with close monitoring due to complications such as tachycardia, hypotension. Nitroglycerin ointment dilates arteries and venous, increases capillary blood flow, and reverses ischemia and necrosis. The mentioned methods show that, when necrosis is established, these methods are useless, and besides timing of recognizing the injury may be delayed in neonates [30]. The immunocompromised state of the neonate demands rapid healing. Acute wound healing consists of four stages: hemostasis, inflammation, proliferation, and tissue remodeling. AM is the inner layer of the placenta that has many biologic and immunologic properties and can induce healing by several mechanisms.AM induces anti-inflammatory, antifibrotic, and antimicrobial properties [31]. These mechanisms are induced by transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), fibroblast growth factors (FGFs), and platelet-derived growth factors (PDGFs) [32]. Amniotic membrane's mesenchymal stem cells and epithelial cells contain a variety of mediators such as epidermal growth factor (EGF), keratinocyte growth factor (KGF), primary fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) [33], which leads to cell proliferation, epithelization, inhibition of fibrosis, inflammation and bacterial infection [34]. Researchers have shown class I and class I b antigens in epithelial cells, mesenchymal cells, and fibroblasts.

In contrast, others have concluded that amniotic epithelial and mesenchymal cells reduce the secretion of HLA class A, B, DR molecules, CD-40, CD-80, and CD-86 stimulatory molecules. So, they do not produce immune responses, which is why there have been no reports of immunogenic reactions due to amniotic membrane use in 100 years [35]. FGF-b is a pro-angiogenic factor and plays an essential role in forming

fibroblast proliferating granulitic tissue [1]. The presence of platelet growth factor (PDGF) and vascular endothelial-derived growth factor (VEGF) in the amniotic membrane suggests a pro-angiogenic role in the hemostasis phase of wound healing [36]. The use of amniotic membrane on the wound showed many beneficial effects on wound management such as analgesic, anti-scar, preventative effect on dehydration, and excessive fluid loss [37].

The TGF- β family is responsible for synthesizing and depositing extracellular matrix (ECM) proteins and regulating and transporting fibroblasts into myofibroblasts [38]. Mesenchymal stem cells (MSCs) can inhibit TGF-β and cause overproduction of fibrosis and scarring [39]. Amniotic epithelial cells contain interleukin-10 (IL-10), promoting the expression of Th1 cytokine cells, MHC class II complexes. IL-10 also increases B-cell survival, proliferation, and antibody production and has been shown to inhibit the production of anti-inflammatory cytokines such as INF-γ, IL-2, IL-3, tumor necrosis factor (TNF-α). Other anti-inflammatory agents such as IL-1 receptor antagonists and tissue metalloproteinase-1, 2, 3, 4 (TIMP) inhibitors have also been found in amniotic cells [40]. Amniotic epithelial cells express intercellular adhesion molecule-1 (ICAM-1) by inflammatory protein cytokines such as tumor necrosis factor (TNF-a) and IL-1b. ICAM-1 increase the uptake and adhesion of leukocytes [41].

Limitations of the study: To date, we could not find AM application in neonatal Extravasation in the literature; with limited cases, we started our work, and we were beware of unwanted complications.

Conclusion:

Implementing an amniotic membrane for treating wounds due to extravasation can be a new approach. This treatment route decreases graft requirement and can be implemented by expert nurses, so in remote NICUs, its usage is easy.

List Of Abbreviations:

AEs(adverse events); AM(amniotic membrane); AMT(amniotic membrane transplantation); bGFG(basic fibroblast growth factor); CTCAE(common terminology criteria for adverse events); ECM(extracellular matrix); EGF(epithelial growth factor); FGF(fibroblast growth factors); ICAM-1(intercellular adhesion molecule-1); IL(interleukin); KGF(keratinocyte growth factor); MSCs(mesenchymal stem cells); NICU(neonatal intensive care unit); PDGFs(platelet-derived growth factors); TGF-β(transforming growth factor-beta); TIMP(the tissue inhibitors of metalloproteinase); TNF(tumor necrosis factor); TSP-1(thrombospondin-1); VEGF(vascular endothelial growth factor)

Declarations:

Funding sources:

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Conflicts of Interest:

The authors declare that they have no conflict of interest.

Availability of data and material:

Not applicable.

Code availability:

Not applicable.

Author contributions:

M Kadivar and K Mirnia designed the study. M Amini and M Afshani designed the trial and analyzed the data. A Hashemi performed the treatment. K Mirnia wrote the manuscript. K Mirnia Supervised the whole study process. All authors read and approved the final manuscript.

Ethics approval:

The Ethics committee of Tehran University of medical science approved the research with the ethical code of IR.TUMS.MEDICINE.REC.1398.530. The project was under the ethical principles and the national norms and standards for conducting medical research in Iran. This study was registered in the Iranian Registry of Clinical Trials (identifier: IRCT20191024045229N2).

Consent to participate:

We explained the treatment steps to the parents, and written consent was obtained from them; also, we asked permission to take a photo of the wound.

Consent for publication:

The consent form has been attached.

Acknowledgements:

The mother of the patient enrolled in this study provided written informed consent for the publication of his case details.

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Figures

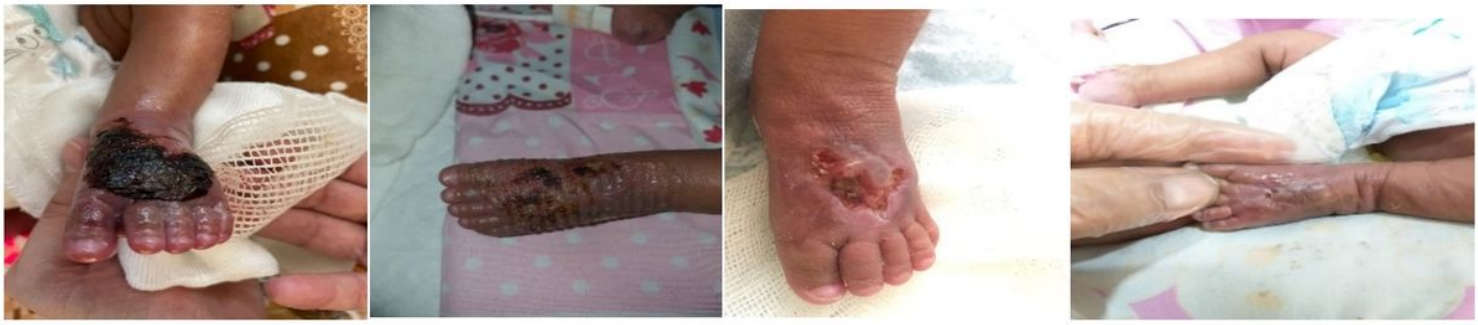

Figure 1

Extravasation stages of treatment with Amniotic Membrane