ORIGINAL ARTICLE

Human dried amniontic membrane (H-DAM) as a biomaterial patch on gastric perforation wound healing: macroscopic evaluation

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ABSTRACT

Introduction: Gastric perforation is a rare occurrence, particularly in neonates. This is an emergency case in this population. The incidence of spontaneous gastric perforation in neonates is 1:2900 live births, with high mortality and morbidity rates. The primary treatment is surgical debridement and repair of the perforation, which has a high incidence of anastomotic leakage. At present, there is a plethora of studies investigating the efficacy of human dried amniotic membrane (H-DAM) technology in promoting wound healing. Consequently, researchers sought to ascertain whether there were differences in the number of adhesion and abscess classifications for the macroscopic evaluation of gastric perforation repair with H-DAM as a biomaterial in New Zealand white rabbits.

Material and Methods: A total of 30 male New Zealand rabbits underwent laparotomy and gastric perforation. These animals were then divided into three groups, with each group comprising 10 rabbits. Group 1 underwent primary repair, group 2 underwent omental patch repair, and group 3 underwent H-DAM patch repair. The rabbits were euthanised on the 7th day and the adhesion score and abscess classification were evaluated.

Result: A total of 30 samples of rabbits were homogeneous. On macroscopic evaluation, it was found that the H-DAM had the lowest mean adhesion score and the lowest incidence of abscess formation compared to all other groups.

Conclusions: It can be concluded that the utilisation of H-DAM as a biomaterial patch in the treatment of gastric perforation in the rabbit model did not result in any instances of leakage, adhesion or infection.

KEYWORDS:
Perforation, gastric, H-DAM

INTRODUCTION

Gastric perforation in neonates is an emergency case in neonatology that has a mortality rate. Gastric perforation in the neonatal period is a rare occurrence. Spontaneous gastric perforation in neonates has an incidence of 1: 2.900 live births, representing 15% of all cases of gastrointestinal tract perforation in neonates and children.[1] The three most common mechanisms causing gastric perforation in neonates are spontaneous perforation, ischaemia and trauma. Gastric perforation caused by iatrogenic trauma is due to the insertion of a nasogastric or orogastric tube. Such iatrogenic perforations are usually located along the major curvature and appear as puncture wounds or short scratches. Traumatic gastric perforation may also occur due to barotrauma during positive pressure ventilation. The ischaemic mechanism of perforation is difficult to elucidate as cases of perforation are associated with severe stressful conditions, such as prematurity, sepsis and neonatal asphyxia.[2,3] Surgery for gastric perforation involves debridement and primary repair. That method has a high incidence of leakage anastomosis in neonate with unstable condition or with poor systemic condition. Gastric perforation has high mortality rate 70%.[4,5]

Currently there are many studies on the use of human dried amniotic membrane (H-DAM) technology for wound healing. Amniotic membrane is the innermost layer of the three layers that make up the placenta. Amniotic membrane has antibacterial properties, low immunogenicity and can help the process of epithelialisation and wound healing.6 Therefore, researchers wanted to prove that there were differences in the number of adhesion and abscess score for the macroscopic evaluation in gastric perforation repair with H-DAM as a biomaterial in New Zealand white rabbits.

MATERIALS AND METHODS

A true experimental study was conducted on 30 samples of rabbits, with none of the subjects dropping out. The inclusion criteria for this study were as follows: New Zealand white rabbit males, aged 6 to 9 months, with a weight of 2 to 3 kg, deemed healthy and active. It should be noted that rabbits were excluded from the study if they had not fasted for a minimum of 12 hours. During the 12-hour fasting period, the subject exhibited aggressive behaviour, attacked other rabbits, and developed a surgical site infection. The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 25.0 for Windows.

A total of 30 experimental rabbits were divided into three groups. Each group consists of 10 rabbits, the first group is primary repair group as control group (K), the second group was amniotic membrane group (A) and the last group was

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Table I: Adhesion score

	Criteria	Score points
Adhesion score	Are uterus, small intestine or omentum attached to the anastomosis? Is any other organ attached to the anastomosis? Feasibility of removing the adhesions bluntly with a swab	1 point per adherent organ 0 = no 1 = yes 0 = no adhesions in the first place 1 = all adhesions can be removed bluntly 2 = only part of the adhesions can be removed bluntly 3 = no adhesions can be removed bluntly at all

Table II: Abscess classification

	Classification	Criteria
Abscess classification	A	No macroscopically visible abscess formation (note That this does not preclude micro-abscesses visible in histopathological examination of the tissue)
	B	Limited, macroscopically visible abscess ("macro-abscess" for short)
	C	Disseminated peritonitis and/or free anastomotic leakage and/or intestinal perforation

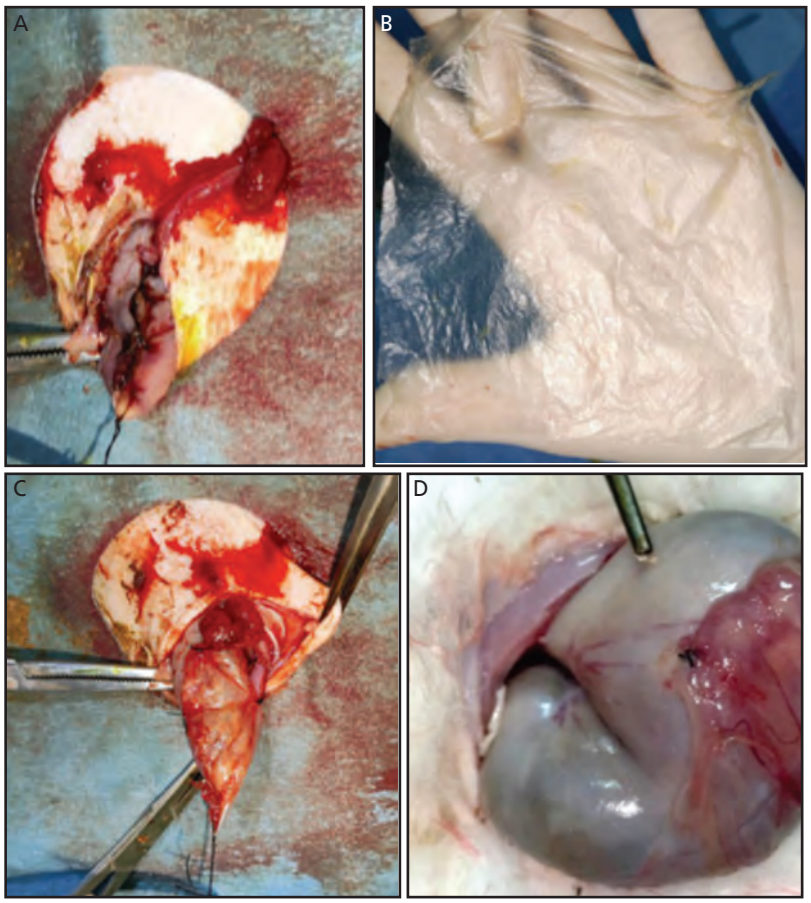

Fig. 1: A. Rupture in the gastric corpus was carried out by primary suturing, B. H-DAM measuring 10×10 cm, C. treatment group by placed H-DAM over primary sutures, D. treatment group by patching omentum over primary sutures.

omental patch group (O). In all three groups, a 2 cm long by 2 cm wide slice with a depth of the entire gastric wall was made in the gastric corpus and perforation repair was performed with 4 to 6 interrupted sutures using 4/0 non-absorbable silk multifilament thread. In group A, the repair was covered with a 4×2 cm wide H-DAM (H-DAM was sourced from the Tissue Bank at Dr. Soetomo Regional General Hospital in Surabaya, Indonesia. It was originated from human placental tissue that underwent a sterile drying process), with the basement membrane facing the serosa surface of the gastric corpus. H-DAM was fixed to the gastric serosa with 2 to 3 sutures. In group O, the repair was closed using the existing omentum in rabbits with the modified Graham Patch technique, namely omentopexy was

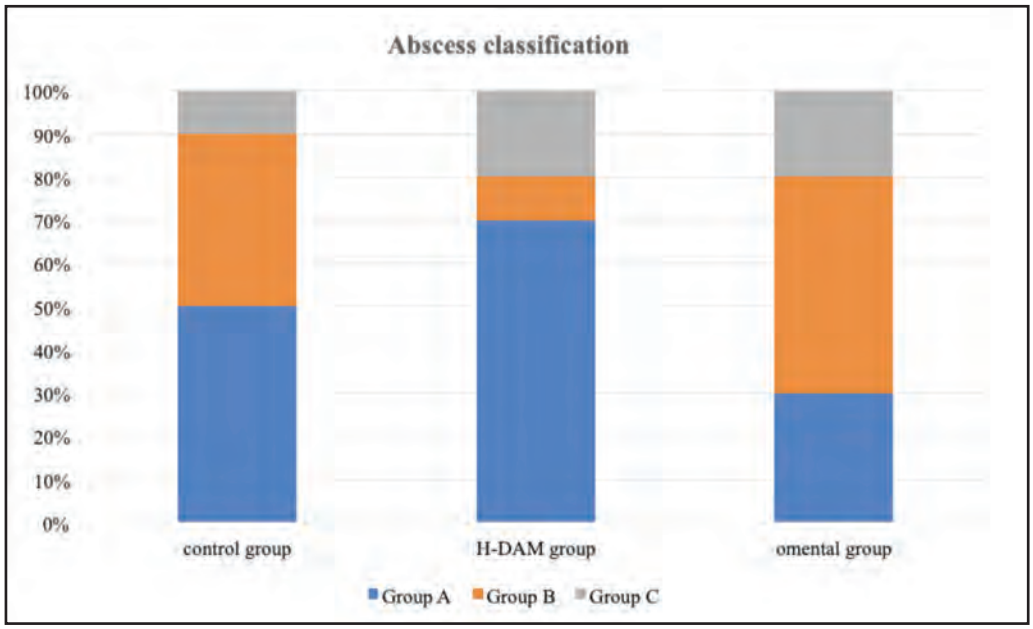

Fig. 2: Abscess classification of the control group : Group A had five animals, Group B had four animals, and Group C had one animal. Abscess classification of the H-DAM group: Group A had seven animals, Group B had one animal, and Group C had two animals. Abscess classification of the omental group: Group had three animals, Group B had five animals, and Group C had two animals.

performed by sewing the omentum facing the surface of the gastric serosa using silk 4/0 2 to 3 stitches. The abdominal muscle layer and skin are closed separately with silk 3/0 sutures in a straight line.

All samples will be terminated on postoperative day 7. Macroscopic evaluation was carried out by looking at the adhesion score and abscess classification on surgical site in each group based on the scores in Tables I and II. The data was then recorded and statistically analysed using SPSS 25 program.

RESULTS

A total of 30 samples met the research requirements, and no rabbits died during the study. The subjects were rabbits aged between 6 and 9 months, with a median age of 7 months in the control group, treatment group with H-DAM and treatment group using omental patches. The Lavene test based on age was employed to assess the homogeneity of the data. The resulting Sig value (0.934) exceeded the 0.05 threshold, indicating that the data were homogeneous. All research rabbits were male (100%), thus ensuring homogeneous data with respect to gender.

All samples were evaluated macroscopically on postoperative day 7 using a scoring system. The next step is to proceed with the one-way ANOVA test in order to analyse the mean adhesion score in each group. The statistical analysis employed non-parametric tests, notably the Kruskal-Wallis test, to assess the mean adhesion score across groups. The Asymp. Sig. 0.415 (>0.05) outcome indicates no significant difference in mean adhesion scores among the groups. The average adhesion scores are: control group (1.7), H-DAM group (1.4), and omental group (2.5). However, the group's mean adhesion scores reveal that the H-DAM group has the lowest score compared to all other groups

The H-DAM group showed the highest occurrence of group A abscesses (70%) compared to the control and omental groups, suggesting it had the least incidents of leakage and secondary infection. Group C (indicating peritonitis and/or anastomosis leakage) was present across all groups, with one in the control group, two in the H-DAM groups, and two in the omental groups, as depicted in Figures 2.

DISCUSSION

Macroscopic observations were conducted during surgical wound reopening in the H-DAM group shown in Figure 2. The H-DAM used for repair had fused with the gastric tissue without any adhesion with the surrounding tissue. The omentum remained unaffected, and there was no tissue leakage after repair.

In measuring abscess classification, seven (70%) rabbits in the H-DAM group did not form abscesses, with five (50%) in the control group and three (30%) in the omental group. This is due to the anti-inflammatory and antibacterial abilities of H-DAM, leading to a significantly lower incidence of anastomosis leakage and abscess formation when compared to the control and omental groups.

Gastric repair is characterised by multiple systemic, local and operative factors that collectively influence the continuum of wound healing. Intraperitoneal infection disrupts the wound healing process by prolonging the inflammatory phase and causing increased expression of tissue proteases.[8]

The antibacterial properties of amniotic membrane are made possible by elements such as interferon, lysozyme, transferrin, progesterone, immunoglobulins and B1c/Bla globulins present in the H-DAM layer. Dried amniotic membrane also reduces exudate by adhering tightly to the

wound. Dried amniotic membrane is also anti-inflammatory due to its abundance of compounds that inhibit inflammatory mediators such as proteases. Dried amniotic membrane contains high molecular weight collagen and growth factors that can facilitate epithelial cell proliferation, induce epithelial differentiation and prevent cell apoptosis. The anti-inflammatory action of H-DAM suppresses pro-inflammatory cytokines IL-1α and IL-β and produces natural metalloprotease (MMP) inhibitors. Its effects actively suppress T lymphocyte proliferation and inhibit monocyte differentiation. The amniotic membrane has natural inhibitors in its matrix that are able to stabilise matrix metalloproteinase expression in inflammatory environments, which plays an important role in the healing process.[8-11]

One of the challenges in this study is the postoperative care stage due to the numerous factors that can affect wound healing. A reduction in appetite after surgery can adversely affect wound healing in some rabbits, so it is vital to quarantine them before and after surgery to achieve the best possible results.

CONCLUSION

The use of H-DAM as a biomaterial patch for gastric perforation is expected to improve the healing process of gastric perforation compared to primary repair of gastric rupture without H-DAM. We conclude that the use of H-DAM as a biomaterial patch on gastric perforation in the rabbit model did not show leakage, adhesion and infection.

Further observations are necessary, especially under microscopic examination, to determine the collagen density, the number of fibroblasts, and other factors that affect the healing process, such as neovascularisation and inflammatory mediators, in repairing gastric perforation using H-DAM as a biologic dressing compared to omental patches in rabbit models. The researchers hope that this study will serve as a reference for further research in humans, particularly in children and infants.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICAL CLEARANCE

Ethics approval was obtained from the Animal Care and Use Committee (ACUC), Universitas Airlangga, Surabaya, Indonesia, with number 2.KEH.143.09.2023 before the study was conducted.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

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