Original Article

Study of Bacterial Contamination in the Amniotic Membrane Obtained For Skin Graft

Sunil Thapa[1], Niran Maharjan[2], Apar Lamichhane[2], Pashupati Babu Pokharel[2], Peeyush Dahal[2], Shankar Mani Rai[3]

[1]Department of Plastic Surgery, Nepal Medical College and Teaching Hospital, Kathmandu, Nepal.
[2]National Academy of Medical Sciences, National Trauma Center, Kathmandu, Nepal.
[3]Kirtipur Hospital, Kathmandu, Nepal.

ABSTRACT

Introduction: Limited availability of skin substitute is one of the main factor that hinders optimal management of the burn patients especially those with larger burns. Amniotic membrane is one of the most easily available and cost effective skin substitute but the risk of bacterial infection and disease transmission are present. Therefore, the aim of this study was to investigate bacterial contaminants associated with amniotic membranes and also to monitor our decontamination process for its effectiveness in decreasing bacterial load.

Methods: This study was conducted in Department of Burns, Plastic and Reconstructive Surgery, National Academy of Medical Sciences, Kathmandu. After normal delivery or Cesarean section delivery total 76 placenta (38 each group) were retrieved and amniotic membrane was harvested from it and sent for culture sensitivity (sample 1) then amniotic membrane was washed with normal saline and small tissue from amniotic membrane was sent for culture and sensitivity (sample 2). After normal saline wash further wash was done with Antibiotic mix solution (normal saline 15ml + Inj. Gentamicin 80mg+cefazolin 1gm + Glycerin 85ml) in a biosafety cabinet class iii chamber (Micro-FiltR India) for 30 minutes. After antibiotic wash amniotic tissue were sent for culture (sample 3).

Results: After use of antibiotics mix solution to wash amniotic membrane marked decreased in bacterial culture was seen in both the groups (36 Cesarean section and 29 vaginal delivery) (p=0.02). Increased bacterial growth was seen in vaginal delivery group with predominance of E. coli but in Cesarean section group, Coagulase negative staphylococcus bacteria were isolated both being sensitive to many drugs like Amikacin, Cefotaxime and Ciprofloxacin.

Conclusion: Bacterial load of amniotic membrane can be reduced by washing with normal saline and antibiotic solution. Due to increased bacterial growth in vaginal delivered amniotic membranes, we suggest only to use amniotic membranes harvested cesarean sections

Keywords: *Amniotic membrane, Burn, bacterial contamination*

Introduction

Burn injury is a public health problem and a significant cause of morbidity and mortality throughout the world. Burn injuries present a particularly significant challenge in Nepal, as they account for more than 55,000 injuries and 216 lost disability-adjusted life years per 100,000 patient-years annually.[1]

Traditionally the treatment of burn is done by daily cleaning the wound, antibiotic dressing and later grafting with autologous split or full-thickness skin graft but it is constrained by the limited available sources, especially in major burns.

Most patients of burn injury in Nepal are from low socio-economic status and are unable to afford the expensive burn treatment. Furthermore, lack of skin substitute to cover the excised burn wound makes the treatment more challenging in these settings. Therefore, mortality rate for patient with more than 40% total burn surface area almost

Correspondence:

Dr. Sunil Thapa
Assistant Professor
Department of Plastic Surgery
Nepal Medical College and Teaching Hospital
Kathmandu, Nepal
Email: sunilyuvrajthapa@gmail.com
Phone number: 9860432795

approaches 95% even in fully equipped tertiary center with dedicated burn teams in Nepal.[2]

Many have favored the use of amniotic membrane (AM) in burns as it is easily available to harvest, promotes wound healing, also shown some antimicrobial effects, pain relief, reduction of fluid requirement and favorable scar formation and needing less frequent dressing changes when applied on burn wounds.[3,4]

Although amniotic membranes are easily available, use of amniotic membranes possesses a risk of bacterial infection and disease transmission.[5] Therefore, bacterial free samples of amniotic membranes are one of the most important concerns for its use.

Therefore, with the help of this study findings we were able to see the pattern of common infectants usually present in amniotic membrane harvested both from caesarean section and vaginal delivery at our centres and also able to monitor our decontamination process for its effectiveness in decreasing bacterial load or eradicating organisms post processing and to our knowledge this type of study hasn't been carried out previously so the outcome of this study may benefit in developing countries like Nepal where the availability of skin graft is not easy or affordable.

Methods

Total of 76 (38 patient in each group) placenta of mothers after delivery either by Cesarean section or normal delivery were included in the study. With precautions and maintaining sterility AM was isolated from placenta and following procedure was undertaken:

At first without any washing, small AM tissue was cut, kept in brain heart infusion broth (BHI) bottles and sent to lab for culture and sensitivity test after labeled as sample.[1]

Second phase same AM was washed with normal saline for multiple times until shiny surface of AM achieved and again small tissue in BHI bottle was sent for culture and sensitivity test labeled as sample.[2]

After saline wash, in third phase same AM was again washed with antibiotic solution containing (normal saline 15ml + Inj. Gentamicin 80mg+cefazolin 1gm). Following antibiotic wash, AM was further processed in a biosafety cabinet class iii chamber (Micro-FiltR India) with high efficiency particulate air (HEPA) filters with high energy Ultra violet (UV) for 30 minutes. Then was sent for culture sensitivity test in BHI bottle and was labeled as sample.[3]

At laboratory amniotic membrane culture and its sensitivity report were provided after 72 hours but if doubt then only was reported by fifth day of sample collection.

Descriptive statistical analysis was done from the demographic data of patients. Results were expressed as mean ± SD. The Chi-Square test will be used to compare discrete variables. Continuous variables were compared using Student's t test for normally distributed variables.

Results

A. Culture positivity of amniotic membranes before wash (Fig 1)

Sample 1		Group		Total	p value
		CS	VD		
Culture positivity of amniotic membrane	N	8(21.1%)	0	8(10.5%)	0.006
	Y	30(78.9%)	38(100%)	68(89.5%)	
Total		38	38	76	

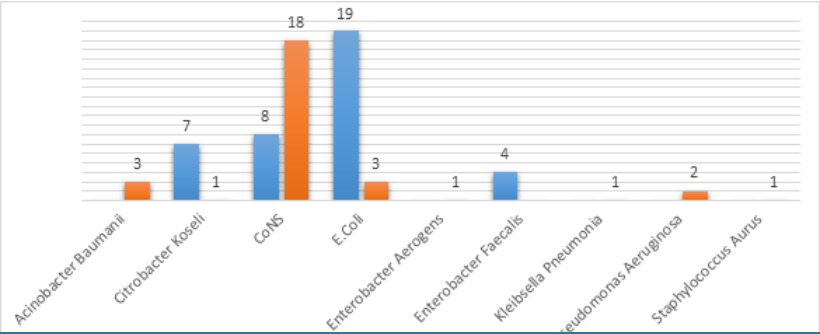

Fig 2: Bacteria isolated in sample 1

B. Bacteria isolated in sample 2 names with chart(fig 3)

Sample 2		Group		Total	p value
		CS	VD		
Culture positivity	N	24(63.2%)	3(7.9%)	27(35.5%)	0.001
	Y	14(36.8%)	35(92.1%)	49(64.5%)	
Total		38	38	76	

C. Bacteria isolated in sample 3 names with chart(fig 5)

Sample 3		Group		Total	p value
		CS	VD		
Culture positivity	N	36(94.7%)	29(76.3%)	65(85.5%)	0.022
	Y	2(5.2%)	9(23.7%)	11(14.5%)	
Total		38	38	76	

Discussion

Despite being a public health problem, the treatment of burn in Nepal has been suboptimal. Limited availability of skin substitute is one of the main factors that hinders the optimal management of the burn patients especially those with larger percentage of burns.[6] Amniotic membranes are one of the most easily available and cost effective skin substitutes.

Therefore this study tried to find out the contamination of amniotic membrane harvested either through VD or CS and how the processing method will reduce the contaminants. The result of this study may help in increasing the effectiveness and safety of amniotic membrane in burn wound coverage.

Use of Daikin's solution, sodium bicarbonate, lyophilization and air drying has been proposed in processing and sterilizing AM.[7,8] Although solely use of isotonic normal saline to wash and its effectiveness in decreasing the bacterial load in AM has not yet been reported, we used isotonic saline for the first wash and then the antibiotic mixed isotonic saline for the second wash. With this easy and readily available technique, we could achieve around 94% sterile AM in CS group while only 76% in VD group (fig 3)

In this study 89% of AM(sample 1) were found to be contaminated (CS 30 Vs 38VD) (fig 1) but the rate of contamination is lower than the previous reported studies as 100 %.[9] This decrease of contamination in our opinion may be due to prior education of the staff concerned for the AM harvest and processing which other authors had promptly advised to focus on but had failed to achieve in the time of need.[10]

In contrast to other studies[9,10] a total of 9 different bacterial species were identified with predominance of Coagulase Negative Staphylococci species (CoNS) in CS group. There is always a chance of increase contamination risk via donor or personnel normal flora and intra operative contamination in CoNS.[11,12] Thus, we may presume that in later days contamination of AM with bacteria like CoNS can be controlled in some extent during cesarean section delivery if good preventive and aseptic measures are assured.

But E. coli being normal inhabitant of the vagina, with higher transmission rate in amniotic fluid, either after membrane rupture or at the time of delivery.[13] It is quite challenging for us to collect VD samples free of contaminations so thus in future we may expect to achieve these but 100% contamination free tissue cultures are yet to be reported.

In our study although different groups of bacterial growth were seen in both CS and VD amniotic membrane , they were sensitive to many drugs like Amikacin, Cefotaxime, Ciprofloxacin, Cotrimoxazole, Levofloxacin, Piperacillin, Gentamicin, Polymyxin B, Tigecycline and Colistin. Thus, on the basis of these findings in future we can use these antibiotics which are more potent and effective during our initial AM washing time to alter our outcome even in vaginal delivered sample.

In spite of handling placenta in sterile operation theatre with consideration of all the aseptic protocol in elective Cesarean section by a single person, same protocol could not be established in normal vaginal delivered placenta since the delivery time was unpredictable and different staff were assigned to receive delivery at different course of time.

Although studies have shown culture of aerobic, anaerobic bacteria and fungi in both normal vaginal and cesarean section delivered harvested amniotic membranes, we

were not able to test for anaerobic and fungi because at present we lack this testing facility at our centre and are expensive but we hope in near future we could test for all the pathogens which may further guide us to change our decontamination process.

Conclusions

Amniotic membrane could be one of the excellent substitutes to cover the excised burn wound. Harvesting, processing and application of the amniotic membranes is rather easy with no morbidity. Usage of simple decontamination procedure like normal saline and antibiotic wash, near total bacterial free amnion sample could be achieved.

Different groups of bacterial growth were seen in amniotic membrane and were sensitive to many drugs thus according to sensitivity pattern we can use antibiotics which are more potent and effective during decontamination process to decease the bacterial load. To improve recipient safety, we suggest only to use AM harvested from Cesarean sections and any tissue which has high suspicion of contamination prior to harvest should be discarded.

References

1. Cai L, Long C, Karki B, Nakarmi K, Iqbal A, Casertano M, et al. Creation of Nepal's First Skin Bank: Challenges and Outcomes. Plast Reconstr Surg Glob Open. 2017;5(11):e1510.
DOI: 10.1097/GOX.0000000000001510
PMID: 29263946 PMCID: PMC5732652

2. Tripathee S, Basnet SJ. Epidemiology and outcome of hospitalized burns patients in tertiary care center in Nepal: Two year retrospective study. Burns Open. 2017;1(1):16-9.
DOI: 10.1016/j.burnso.2017.03.001

3. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater. 2008;15:88-99.
DOI: 10.22203/eCM.v015a07
PMID: 18446690

4. Kesting MR, Wolff KD, Hohlweg-Majert B, Steinstraesser L. The role of allogenic amniotic membrane in burn treatment. J Burn Care Res. 2008;29(6):907-16.
DOI: 10.1097/BCR.0b013e31818b9e40
PMID: 18849850

5. Marangon FB, Alfonso EC, Miller D, Remonda NM, Muallem MS, Tseng SC. Incidence of microbial infection after amniotic membrane transplantation. Cornea. 2004;23(3):264-9.
DOI: 10.1097/00003226-200404000-00008
PMID: 15084859

6. Rai S, Karki B, Nakarmi K, Gharti Magar M, Nagarkoti K, Joshi K, et al. Retrospective study on early outcome of acute burn injuries treated at Nepal Cleft and Burn Centre of Public Health Concern Trust-Nepal. Journal of Nepal Health Research Council. 2014;12:195-9.

7. Ganatra MA, Durrani KM. Method of obtaining and preparation of fresh human amniotic membrane for clinical use. J Pak Med Assoc. 1996;46(6):126-8.

8. Jirsova K, Jones GLA. Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review. Cell Tissue Bank. 2017;18(2):193-204.
DOI: 10.1007/s10561-017-9618-5
PMID: 28255771

9. Adds PJ, Hunt C, Hartley S. Bacterial contamination of amniotic membrane. Br J Ophthalmol. 2001;85(2):228-30.
DOI: 10.1136/bjo.85.2.228
PMID: 11159493 PMCID: PMC1723825

10. Aghayan HR, Goodarzi P, Baradaran-Rafii A, Larijani B, Moradabadi L, Rahim F, et al. Bacterial contamination of amniotic membrane in a tissue bank from Iran. Cell and Tissue Banking. 2013;14(3):401-6.
DOI: 10.1007/s10561-012-9345-x
PMID: 23095908

11. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870-926.
DOI: 10.1128/CMR.00109-13
PMID: 25278577 PMCID: PMC4187637

12. Levy MF, Schmitt DD, Edmiston CE, Bandyk DF, Krepel CJ, Seabrook GR, et al. Sequential analysis of staphylococcal colonization of body surfaces of patients undergoing vascular surgery. J Clin Microbiol. 1990;28(4):664-9.
DOI: 10.1128/jcm.28.4.664-669.1990
PMID: 2332464 PMCID: PMC267773

13. Guiral E, Bosch J, Vila J, Soto SM. Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiol Lett. 2011;314(2):170-3.
DOI: 10.1111/j.1574-6968.2010.02160.x
PMID: 21133987