

HUMAN AMNIOTIC MEMBRANE IN DIABETIC FOOT HEALING PROCESSES: A RETROSPECTIVE STUDY

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ABSTRACT

Introduction: Bacterial skin infections represent a complex problem, especially for their degree of diffusion in intra- and extra-hospital environment and are identified and recognized with the term ABSSSI (acute bacterial skin and skin structure infections). The human amniotic membrane HAM, contains several components of the extracellular matrix and a low immunogenicity that characterize an important therapeutic role. AA evaluated the therapeutic role of the human amniotic membrane HAM in chronic infectious processes.

Materials and methods: Sixteen type II diabetic subjects (5 F - 11 M mean age 61±5 years) with chronic ulcerative diabetic foot lesions were evaluated in this retrospective study. All patients were treated with human amniotic membrane prepared using fresh human placenta.

Results: For the clinical evaluation in the post-treatment and recisely after 15 weeks of grafting the following data were found: 56.2% scar in full resolution and the remaining 43.8% partial resolution. Histological evaluation of the explanted grafts showed a single layer of fairly compact skin cells.

Discussion-conclusion: Human amniotic membrane has proven to be an excellent dermal substitute in the treatment of difficult ulcers such as diabetic foot subjects leading to healing and improvement of skin lesions as well as quality of life of patients. These remedies have proven to be quite safe since human amniotic membranes are taken from donors undergoing elective cesarean delivery.

Keywords: human amniotic membrane HAM, bacteria, type II diabetes, diabetic foot.

DOI: 10.19193/0393-6384_2021_3_223

Received November 15, 2020; Accepted February 20, 2021

Introduction

Bacterial skin and soft tissue infections (SSTI) are a nosological entity that includes various diseases of different severity that involve precisely the skin and underlying tissues. The chronicity is due to the fact that healing is difficult or often impossible⁽¹⁻⁸⁾. These infections represent a problem of difficult management, especially for their degree of diffusion in intra- and extra-hospital environment and are identified and recognized with the term ABSSSI (acute bacterial skin and skin structure infections). The bacterial pathogens most frequently responsible for ABSSSIs are Gram-positive cocci and, among them, *Staphylococcus aureus* (S.

aureus) and β -hemolytic streptococci. Other pathogens involved include Gram-negative bacilli, enterococci, and anaerobic bacteria, the latter often in a polymicrobial context. The main culprit is *S. aureus*, involved in approximately 30 to 40% of cases and often methicillin-resistant *Staphylococcus aureus* (MRMRSA⁽⁹⁻²⁶⁾). Frequently isolated and associated with ABSSSI is *Streptococcus pyogenes*⁽²⁷⁾. Antibiotic therapy has progressively lost its clinical efficacy over time due to the spread of the phenomenon of antibiotic resistance that has progressively reduced the therapeutic options. In particular, the etiological spread of MRSA, which is resistant to the action of all conventional β -lactams and often also to other antibiotics anti-staphylococcal antibiotics (e.g.,

macrolides, clindamycin, fluoroquinolones, including ciprofloxacin), has made treatment considerably more difficult. Several studies have evaluated the therapeutic role of the human amniotic membrane HAM in chronic infectious processes⁽²⁸⁻³⁵⁾. In particular, Douglas investigated the use of the amniotic membrane as a temporary biological coating in the coverage of burns.

The extracellular component of this membrane turns out to be composed of several primary biomolecules: specialized proteins (Fibronectin, TIMP and Laminins); proteoglycans; structural proteins (Collagen type I, III, IV, V, VI and elastin). Several are the properties that characterize the MAU as the high capacity of cell regeneration and expression of growth factors (EGF, TGF, FGF, PDGF A and B) and low immunogenicity, for the lack of expression of antigens HLA -A, B, C, or beta2 microglobulin. The antibacterial activity of MAU is reserved by the expression of lactoferrin and IL-1RA both of which have "in vitro" and "in vivo" a remarkable antimicrobial and anti-inflammatory power. On the basis of this knowledge we proposed to evaluate the efficacy of HAM on some subjects with diabetic foot.

Materials and methods

Study design

16 subjects with type II diabetes (5 F - 11 M mean age 61±5 years) with diabetic foot were admitted to our department.

Inclusion criteria are:

- subjects with diabetic foot;
- Absence of allergic.

Exclusion criteria are:

Subjects in the medical therapy with systemic or topical medications or topical steroids during the four weeks preceding the start of the study. The basal characteristics are described in table 1.

Charateristics	n.	Mean ±Standard Deviation	Range
Sex (F/M)	5/11		
Age (years)		64±5	
BMI (kg/m2)		26.9±3.7	
Heart Rate (b.p.m.)		84±12	
Systolic blood pressure (mmHg)		137±15	
Diastolic blood pressure (mmHg)		87±10	
Smokers (yes/no)	3/13		
Plasma glucose (mg/dl)		202±10	
HbA1c % (range)			7.5-9.5
Cholesterol (mg/dl)		241±18	
HDL cholesterol (mg/dl)		38±10	
LDL cholesterol (mg/dl)		177±12	
Triglycerides (mg/dl)		296±12	

Table 1: Basal characteristics.

Evaluation parameters

The clinical and bacteriological response was evaluated according to a careful physical examination of the patient relative to the local signs and symptoms, and tests of bacteriological examinations and laboratory (eradication of bacteria on the skin). After 15 weeks clinical response was evaluated according to the standardization and scarring of the skin ulcer: full scar, partial scar and worsening.

Treatment

Patients included in our study were subjected to the following therapeutic skin swab at enrollment and after local and systemic antibiotics and after 7 days from the last administration antibacterial. Particularly after treatment for at least 20 days with antibiotic tobramycin in local therapy and after its negative culture tests, we proceeded with the addition of the amniotic membrane.

Bacteriological analysis

Testing of the chronic ulcer swab Hess was any carried out to search for aerobic bacteria. Samples from patients were seeded in the appropriate culture medium and incubated in aerobic for the isolation and identification of bacteria⁽³⁶⁻⁴¹⁾.

Results

The demographic characteristics of the type II diabetic patients included in our study with cone lesions in the diabetic foot are listed in the following Table 1

Table 2 shows the number and percentage of positivity on bacteriological examination of skin swabs of diabetic foot lesions divided by mono-microbial and poly-microbial findings.

Number of patients	Tests		Mono microbial		Poly microbial	
	n.	%	n.	%	n.	%
(16 pz)	9	56.2	7	43.8

Table 2: Number and percentage of bacteriological positivity of diabetic foot skin swab tests divided into two groups "mono microbial" and "poly microbial".

Table 3 attached shows the number and percentage of bacterial isolates in the skin surface of lesions and "in vitro" activity to tobramycin. After treatment for 20 days with the antibiotic tobramycin in local therapy and after its negative culture tests, we proceeded with the implantation of the human

amniotic membrane. For the clinical evaluation (Table 4) in the post-treatment and recisely after 15 weeks of grafting the following data were found: 56.2% scar in full resolution and the remaining 43.8% partial resolution. Histological evaluation of the explanted grafts showed a single layer of fairly compact skin cells.

Microorganisms	n.	%	Tobramycin		
			I	S	R
S. epidermidis	6	37.5	2	4	-
S. aureus	5	31.3	-	5	-
Morganella morganii	2	12.6	1	1	-
E. coli	1	6.2	-	1	-
P. aeruginosa	1	6.2	-	1	-
E.coloncae	1	6.2	-	1	-
Total strains	16	100	3(18.7%)	13(81.3%)	-

Table 3: Number and percentage of bacterial isolates in the skin surface of diabetic foot lesions and "in vitro" activity to tobramycin.

I= intermediate; S=sensible e R= resistant

number of patients	full scar		partial scar		Worsening	
	n.	%	n.	%	n.	%
(16 pz)	9	56.2	7	43.8	----	-----

Table 4: Clinical response in patients with diabetic foot injury treated with human amniotic membrane.

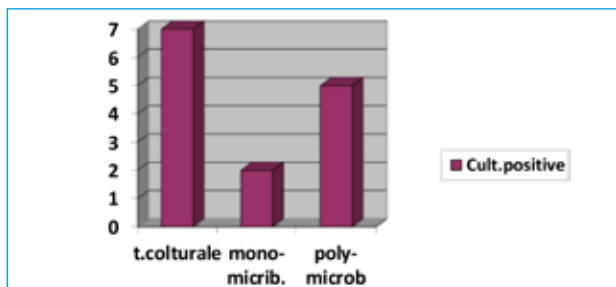


Figure 1: Number of bacteriological positivity of diabetic foot skin swab tests divided into two groups "mono microbial" and "poly microbial".

Figure 1 shows the number of culture positivity of skin swab tests of subjects with diabetic feet and the ratio of overall positive tests to specific monomicrobial or polymicrobial findings.



Figure 2: Diabetic subject with a chronic diabetic foot ulcer.

Figure 2 shows diabetic subject with a chronic diabetic foot ulcer.

Figure 3 shows a subject with diabetic foot after treatment with human amniotic membrane.



Figure 3: Subject with diabetic foot after treatment (3 months) with human amniotic membrane.

Discussion and conclusions

Human amniotic membrane has proven to be an excellent dermal substitute in the treatment of difficult ulcers such as diabetic foot subjects leading to healing and improvement of skin lesions as well as quality of life of patients. These remedies have proven to be quite safe since human amniotic membranes are taken from donors undergoing elective cesarean delivery. In fact, donors are screened at the time of birth of the baby and at 6 months distance (to test for seroconversion) for infectious diseases (including HIV), hepatitis B and C viruses, cytomegalovirus and syphilis. Regarding the culture tests obtained from swabs from chronic lesions of diabetic feet, polymicrobial findings were frequently obtained and this determines a rather difficult interpretation of the result and attribute an etiological role to bacterial isolates that do not represent the true classical pathogens such as S. aureus or S. pyogenes. In SSTI of diabetic foot, microbiological analysis can be useful and decisive to indicate the antibiotic therapy, but unfortunately often this therapeutic solution is ineffective⁽²⁰⁻²²⁾.

The data of sensitivity to antibiotics of the isolated microorganisms was useful for the therapeutic choice, to confirm the appropriateness of a specific therapeutic choice with tobramycin⁽²⁹⁾. Based on these considerations, a natural therapy such as human amniotic membrane can certainly represent a really important therapeutic remedy in these chronic infectious processes. It is clear that our case history is quite limited and should be confirmed and extended through a larger number of treated subjects, in fact the data obtained by us are sufficient to identify the way to follow to treat these important chronic diabetic foot lesions. The related psychological aspects have been treated by Dr. Chisari Eleonora Margherita.

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