Journal of Robotic Surgery (2019) 13:475–481
https://doi.org/10.1007/s11701-018-0873-7

ORIGINAL ARTICLE

A matched and controlled longitudinal cohort study of dehydrated human amniotic membrane allograft sheet used as a wraparound nerve bundles in robotic-assisted laparoscopic radical prostatectomy: a puissant adjunct for enhanced potency outcomes

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Received: 21 August 2018 / Accepted: 9 September 2018 / Published online: 12 September 2018

Abstract
We sought to explore the potency outcomes in two systematically controlled, non-randomized, matched, homogenous patient cohorts, which either underwent intervention (INT) with placement of dehydrated human amniotic membrane (dHAM) around nerve bundles (NVB) during robotic-assisted laparoscopic radical prostatectomy (RALP) or did not (CON). It is hypothesized that dHAM use would lead to better potency outcomes. 1400 eligible informed, consented patients underwent full bilateral nerve-sparing RALP by a single surgeon, wherein 700 patients had dHAM allograft wrapped around the NVB and 700 did not. Groups were matched demographically, clinically, and biochemically. Potency was defined as the ability to have satisfactory penetrative intercourse > 50% of time with SHIM score of ≥ 17 with or without of phosphodiesterase-5 inhibitors. A retrospective matched longitudinal cohort study was performed at 1 year. The first noticeable erection sufficient enough for a satisfactory penetrative intercourse was significantly earlier ($p < 0.01$; 34.6 ± 3.6 days), whereas the decrease in SHIM score was lower (4.27 ± 0.14 days) in INT. Binary logistic regression demonstrated that INT was an independent significant ($p < 0.001$) predictor of achieving potency at 1 year, such that INT was 3.86 times (95% CI 2.43–6.13) more likely to achieve potency in the same period when compared with CON. Chi square analysis demonstrated that recovery of potency in INT was better ($p < 0.05$) in every quarter compared to CON. A higher ($p < 0.005$) percentage (93.1%) of INT regained potency versus CON (87.1%) at 1 year.

Keywords Prostatectomy · Amnion · Allografts · Longitudinal study

Abbreviations

SHIM	Sexual Health Inventory for Men Questionnaire
NVB	Neurovascular bundles
RALP	Robot-assisted laparoscopic radical prostatectomy
dHAM	Dehydrated human amniotic membrane

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Introduction

Quality of life after RALP, especially with regards to potency, is a major concern for patients undergoing surgery. It is thus imperative that surgeons adopt surgical techniques to minimize detrimental effects on potency postoperatively. Prolonging life spans with a younger age at diagnosis makes the potency aspect gain paramount importance. Sixty-eight percent of sexually active men without prostate cancer were willing to trade in 10% of their life span if it gave them the pre-operative degree of sexual potency post procedure [1]. Conjuring this desired result is challenging in view of various recognized and unrecognized procuratorial peri-operative factors. Importantly, neuropraxia and stretch injury to the cavernosal nerves contributes to the delayed and often reduced potency outcomes. Concerted intra-operative confounding factors like amount and quality of nerves spared, atraumatic and correct plane of dissection, local cooling,

thermal dissection, and lateral traction, all have been proven to result in diminished sexual functioning later [2–5]. Use of dehydrated human amnion membrane (dHAM) has been touted to be effective in improving potency outcomes post RALP along with its long-standing use in various surgical procedures like treatment of chronic venous stasis ulcers, diabetic wounds and burn injuries [6, 7]. dHAM is a composite mechanically robust, biologically active amniotic tissue membrane derived naturally from consensually donated human placenta of screened and tested women obtained during a C-section according to the American Association of Tissue Banks standards and is considered a tissue product under section 361 of the Public Health Service Act. For medicinal use, it is minimally manipulated, cleaned, dehydrated and sterilized and is available in sheets, wraps, particulate, and membrane configurations. Its safety for human use has been documented by Ogaya et al. [8]. dHAM augments critical healing steps by preventing fibrosis, in addition to causing neoangiogenesis [9, 10]. dHAM wrapped around severed cavernosal nerves, in rats, exhibits better mating response to electrical stimulation [11]. We envisaged extrapolating this effect of placenta-derived membranes to human cavernosal nerve recovery in the current study. Despite many methods reported in literature, no technique has been unanimously accepted nor rejected to minimize the nerve cell damage during RALP and augment potency recovery later [2–5, 12]. This is an important gap in knowledge. Accordingly, this study was undertaken with the prime objective to quantify dHAM's impact in terms of time taken to return to predefined degrees of potency and the secondary objective being to assess the quantal change in potency at 3 monthly intervals until 1 year post RALP, subjectively manifested as difference in sexual health inventory for men (SHIM) questionnaire score, in the two study groups. We hypothesized that the use of dHAM as a nerve wrap around the neurovascular bundles during full bilateral nerve-sparing RALP would lead to early return of potency with significantly lesser quantal decline of the same over 1 year follow-up.

Materials and methods

Materials

This is a retrospective analysis of 1400 consented patients selected from 1540 consecutive patients undergoing RALP at a high-volume center. They underwent RALP by the standard trans-peritoneal six port technique using the da Vinci surgical system Si (Intuitive Surgical, Sunnyvale, CA, USA) along with or without the application of dHAM allograft nerve sheet as a wrap (Amniofix™ of size 6 cm × 6 cm; BU-5660, MiMedx Group Inc, Marietta, GA, USA), by a single surgeon who does more than 200 RALPs per year. To maintain consistency in the study, we used the same placental product, Amniofix™, despite many varieties available commercially.

Exclusion criteria

Absolute patient numbers are in parenthesis. Age ≥ 75 years (34), salvage procedures (3), SHIM score < 17 (29), history of coronary artery bypass grafting (4)/angioplasty (14) or peripheral vascular disease (3), as they were independent exclusion criterion, PSA > 10 ng/ml (12), disease staged cT3 (32), and patients lost to follow-up within 1 year (9).

Surgical procedure and methodology

All potent patients as defined by a minimum SHIM score of 17 and able to have satisfactory penetrative intercourse more than 50% of the time with or without use of PDE-5 inhibitors were included in the study. 700 patients (the intervention group, INT) underwent full athermal intrafascial clipless complete bilateral nerve-sparing RALP, a minimum of 6 weeks after the biopsy, with dHAM allograft wrapped around the neurovascular bundles before the Van Velthoven anastomosis through an assistant port. Small bleeding points were coagulated with judicious use of spot electrocautery at a low setting or with a suture placed meticulously to control the bleeding point to minimize any nerve injury. Maximum urethral length preservation as advocated by the principal author of the present study was done for continence control, Hamada et al. [13]. 700 patients matched for age, body mass index, AUA score, prostate specific antigen, clinical stage, baseline sexual function, Charlson comorbidity index and nerve-sparing status but without the allograft usage, operated within the same time frame as INT formed the control group (non-intervention control group, CON). The procedure in all the patients was performed by a single surgeon, no residents or fellows were involved. Group allocation of eligible patients was not random but systematic. Charlson comorbidity index was used in this study to negate the atherosclerotic effects of smoking, hypertension, diabetes and hyperlipidemia. Every eligible alternate patient was proffered the allograft, to eliminate the self-selection bias. Comparable demographic, clinical, biochemical and peri-operative parameters are presented in Tables 1 and 2. Patients from both groups were discharged on the first post-operative day and were initiated on a penile rehabilitation program, wherein they received a PDE-5 inhibitor every night (Tadalafil 5 mg) starting 3 weeks after surgery and a vacuum erection device or alprostadil injection/urethral suppository twice a week starting 4 weeks after surgery for the duration of the study. Compliance to this

Table 1 Preoperative parameters of all patients

Parameter	CON	INT	*p* value
Number of patients	700	700	
Age			
Mean ± SD	66.7 ± 4.5	67 ± 4.4	< 0.001
Median	67	67	
Body mass index (kg/m^2)			
Mean ± SD	26.6 ± 3	26.8 ± 3	< 0.001
Median	26.8	26.8	
Pre-op SHIM score			< 0.001
Mean ± SD	23.3 ± 1.6	22.9 ± 1.6	
Median	23	22	
AUA score			0.423
Mean ± SD	5.9 ± 3.2	6 ± 2.5	
Median	6	6	
PSA			< 0.001
Mean ± SD	5.5 ± 2	5.7 ± 1.8	
Median	5.4	5.5	
Clinical stage (%)			0.057
T1a-T1b	16 (2.3)	24 (3.4)	
T1c	648 (91.4)	603 (86.1)	
T2a	22 (3.1)	38 (5.4)	
T2b	17 (2.4)	27 (3.8)	
T2c	5 (0.7)	8 (1.1)	
Gleason score pre-op (%)			
≤ 6	529 (75.6)	557 (79.6)	
7	160 (22.9)	136 (19.4)	0.104
≥ 8	11 (1.6)	7 (1)	
D'Amico Risk (%)			0.299
Low risk	517 (73.9)	533 (76.1)	
Medium risk	167 (23.9)	154 (22)	
High risk	16 (2.3)	13 (1.9)	

SD standard deviation, *SHIM* sexual health inventory in men, *AUA* American Urological Association

Table 2 Intra- and post-operative parameters of all patients

Parameters	CON	INT	*p* value
Console time (min)			0.009
Mean ± SD	67.3 ± 9.7	66.5 ± 13.3	
Median	66	68	
Estimated blood loss (ml)			
Mean ± SD	99.8 ± 49.8	86 ± 44.3	0.393
Median	90	100	
Prostate weight			0.019
Mean ± SD	47.8 ± 3.2	49.4 ± 14.9	
Median	45	47	
Post-op SHIM score			< 0.001
Mean ± SD	18.2 ± 3.1	18.5 ± 2.8	
Median	19	19	
Clavien-Dindo classification			
Grade 1	151	104	
Grade 2	4	1	
Grade 3	0	0	< 0.001
Grade 4	0	0	
Grade 5	0		
Post-op Gleason score			0.020
≤ 6	404 (57.7)	444 (63.4)	
7	251 (35.9)	229 (32.7)	
≥ 8	45 (6.4)	27 (3.8)	
Positive surgical margins			0.007
< 1 mm (%)	46 (6.5)	35 (5)	
1–2 mm (%)	16 (2.3)	3 (0.4)	
> 2 mm (%)	2 (0.3)	0 (0)	
Extra capsular extension (%)	64 (9.1)	38 (5.4)	< 0.001
Seminal vesicle invasion (%)	5(0.7)	3 (0.4)	0.314
Lymphovascular invasion (%)	30 (4.3)	48 (6.8)	0.036
Perineural invasion (%)	140 (20)	146 (20.9)	0.064
Metastatic pelvic lymph node	00 (00)	00 (00)	
Potency rates at 1 year	87.1%	93.1%	< 0.001
PSA at 1 year > 0.1 (%)	14 (2)	10 (1.4)	< 0.01

SD standard deviation

program was periodically monitored by the office technicians telephonically. Subsequent potency status evaluation in both groups, was done at regular 2-week telephonic interview and 3-month clinical interview wherein the patients reported the date they had their first erection sufficient enough to have penetrative intercourse, if they had one, along with the SHIM score. The patient filled out forms on every visit and data were recorded. Out of state/country patients (31 in INT, 26 in CON) and patients who missed their scheduled office appointment (19 in INT, 22 in CON) were interviewed telephonically to assess compliance and their degree of potency on the due date. Every patient in both the groups underwent PSA monitoring at 3-monthly interval. Biochemical recurrence was defined as PSA level of > 0.1 ng/ml. Data was progressively accumulated in a customized data base and then analyzed retrospectively to compare both the groups.

Statistical analysis

Statistically, the confounding variables of the two groups were similar and differed only in terms of the study variable dHAM retaining the validity of the study. Binary logistic regression (Table 3) was used to examine the relationship between independent variables including Age, BMI, AUA score, ASA Score, PSA, prostate weight, pre- and post-operative Gleason's score, console time, estimated blood loos, clinical staging, D'Amico risk levels, and dHAM usage on potency at 1 year in patients undergoing

Table 3 Binary logistic regression of outcome erection at 12 months

Variables	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for EXP (B)	
							Lower	Upper
PSA	−0.119	0.049	5.824	1	0.016	0.888	0.807	0.978
AGE	0.002	0.021	0.006	1	0.938	1.002	0.960	1.045
PRE_OP_SHIM	0.394	0.065	37.190	1	0.000	1.483	1.307	1.683
prostate_weight	−0.008	0.007	1.398	1	0.237	0.992	0.979	1.005
PRE_GLEASON	2.378	1.256	3.583	1	0.058	10.783	0.919	126.495
Console_time	0.007	0.008	0.745	1	0.388	1.007	0.991	1.024
EBL	−0.001	0.002	0.284	1	0.594	0.999	0.995	1.003
Clinical_stage			8.558	4	0.073			
Clinical_stage (1)	−6.212	3.170	3.839	1	0.050	0.002	0.000	1.002
Clinical_stage (2)	−6.299	3.119	4.078	1	0.043	0.002	0.000	0.830
Clinical_stage (3)	−4.476	3.252	1.894	1	0.169	0.011	0.000	6.675
Clinical_stage (4)	−5.096	2.214	5.301	1	0.021	0.006	0.000	0.469
D_amico_risk			5.718	2	0.057			
D_amico_risk (1)	6.340	2.945	4.634	1	0.031	566.789	1.764	182161.921
D_amico_risk (2)	4.105	1.770	5.376	1	0.020	60.622	1.887	1947.503
positive_margin	0.266	0.377	0.497	1	0.481	1.305	0.623	2.731
EC_disease	0.020	0.334	0.004	1	0.951	1.021	0.531	1.963
SV_invasion	−0.813	1.123	0.525	1	0.469	0.443	0.049	4.003
Lymphovascular	−0.616	0.360	2.920	1	0.087	0.540	0.267	1.095
Peri_neural	−0.050	0.228	0.048	1	0.827	0.951	0.608	1.488
Claviendindo	−0.121	0.224	0.290	1	0.590	0.886	0.571	1.375
dHAM usage	1.349	0.237	32.531	1	0.000	3.855	2.425	6.129
Constant	−21.137	8.174	6.686	1	0.010	0.000		

RALP to examine the determinants of potency recovery (time to first significant erection) after the surgery. A student's *t* test was done to compare the same continuous variables between the groups. Figure 1 shows the change in SHIM value, ΔSHIM score, (A), time to first erection (B) between the groups. Chi square test was utilized to compare the potency per quarter (3, 6, 9, 12 months) between the two groups. All statistical analyses were performed using SPSS v.22.0 software (IMB, Chicago, IL, USA) by the institutional statistician.

Results

Data are presented as means ± SD unless otherwise specified. Mean time to potency was 95.53 ± 76.2 days in CON and 60.85 ± 61.2 days in INT. The number of patients achieving potency every month was higher in INT than CON throughout the year. Time to potency recovery was significantly less ($p < 0.05$) in INT, while the quantal decrease in the ΔSHIM score was significantly ($p < 0.05$) lower too (Fig. 1). Moreover, INT had a significantly higher percentage of patients becoming potent ($p < 0.005$) compared to CON every quarter

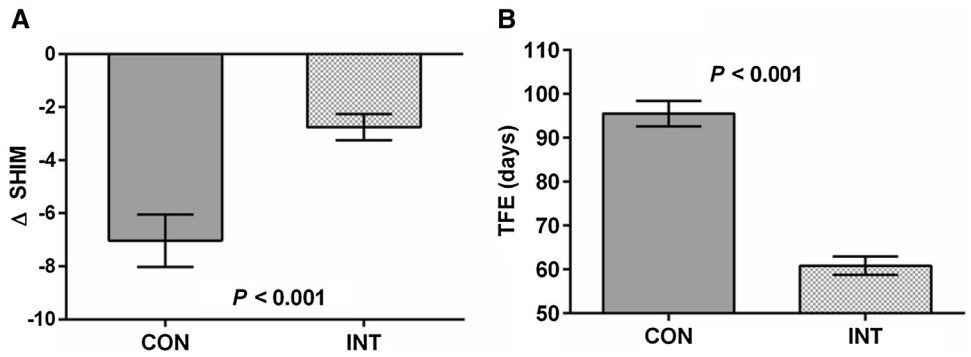

Fig. 1 Quantal change in SHIM value and time for return to potency in CON and INT groups

post-surgery (Table 4). With advancing age, the return to potency was delayed in both the groups, age being an independent predictor of return to potency. Those who regained potency, irrespective of when it was achieved, did not lose it and remained potent until the end of study. Both the console time and estimated blood loss were not statistically different between the two groups. At 3 months post procedure INT had a greater number of patients regaining potency and the average day of return to potency was also lesser than CON. At the end of 1 year, the total number of potent men in CON was 87% and in INT was 93%, the difference being statistically significant ($p < 0.001$), suggesting that the use of dHAM leads to its beneficial effects beginning early in the convalescence period and sustained for more than a year post procedure. dHAM use was not associated with any membrane-related adverse effects or allergic reactions.

Discussion

The goal of the present study was to evaluate and validate the impact of dHAM on the potency parameters post RALP. Notwithstanding the fact that we were the first in the world to have reported the use of dHAM in early return of erectile function post RALP (31st World Congress of Endourology, Taipei, 2014), to the best of our knowledge this is the largest series of 1400 patients. We found that dHAM was associated with better and faster recovery of erectile function after surgery. Moreover, there were higher percentages of patients who became clinically potent in INT (93%) versus. CON (87%) at 1 year. The first noticeable erection enough for a satisfactory penetrative intercourse was significantly earlier ($p < 0.01$; 34.6 ± 3.6 days), whereas the decrease in SHIM score was lower (4.27 ± 0.14 days) in INT. The result of this study suggests that dHAM usage in RALP to wrap around the NVBs can help achieve earlier and better potency outcomes.

Widespread use of dHAM has been marred by the concerns regarding its acquisition, preparation, storage and the potential to spread tissue-borne diseases. Recently though with better collection methodologies, sterile ways of preparing it and storing the membrane as a dehydrated product has rekindled the interest in its use. This kind of processing gives the membrane a long shelf life and preserves its biochemical capability to positively modify the process of wound healing [9]. Therapeutically, dHAM has been utilized in predicaments demanding least inflammation and scarring but more soft tissue regeneration (e.g., in treatment of chronic venous and diabetic ulcers, chronic wounds, etc). dHAM has regenerative cytokines, chemokines, and other growth factors which stimulate migration and proliferation of omnipotent stem and epithelial cells to the site of its application [14].

Formation of perineural adhesions around cavernosal nerves occurs after the surgery because of surgical tissue trauma. This trauma may result from incisive mechanical or thermal injury, ischemia or traction on the nerves [15]. Microscopically, increased penile fibrosis, decreased smooth muscle cell content, and increased lysyl oxidase have all been incriminated for post-operative erectile dysfunction following cavernosal nerve injury during RALP [16]. Like any other injury, cavernosal nerve injury heals with inflammation. Consequently, minimizing trauma, decreasing inflammation along with facilitation of recovery, leads to improved potency outcomes. dHAM has been shown to have less perineural adhesions as compared to the controls when wrapped around nerves [17]. Enhanced cavernosal nerve cell survival and regeneration of axons are credited to the neurotrophic factors present in dHAM.

dHAM has been used as a mechanical barrier to prevent epidural fibrosis thus, making revision surgery easier [18]. dHAM is reported to be associated with complete healing of an irradiation-induced vesicovaginal fistula repaired robotically with no complications at the end of 1 year [19].

The incidence of erectile dysfunction after RALP is incongruently reported. The rates of erectile dysfunction appear to be higher in multicenter, multi-surgeon series compared to single center, single-surgeon series [20]. Herein comes the role of high-volume centers with a single experienced surgeon performing the procedure. In the current

Table 4 Total patients achieving potency per quarter and new patient's average day of attaining potency every quarter

Months since RALP	CON ($n = 700$)		INT ($n = 700$)		
	Total potent, men *n* (%)	A ± SD, median	Total potent, men *n* (%)	A ± SD, median	*p* value of potent men group
3	474 (67.8)	77 ± 74.2, 73	504 (72)	37 ± 69, 50	0.05
6	532 (76)	157 ± 74.7, 72	574 (82)	107 ± 70.6, 51	0.006
9	588 (84)	253 ± 74.3, 73	630 (90)	191 ± 70.3, 50.5	0.001
12	609 (87)	349 ± 78.2, 70	651 (93)	274 ± 75, 60	< 0.001

A is the average day to return to potency

study, the procedures were all done by a single high-volume surgeon, performing more than 200 RALPs every year.

Di Pierro et al. in a prospective study with bilateral nerve-sparing surgery and potency defined like in our study, had a potency rate of 55% at the end of 1 year post RALP [21]. Asimakopoulos et al. with similar potency and age exclusion criteria like our study had a potency rate of 77% at 1 year post RALP [22]. Haglind et al. with similar exclusion criteria and surgeries performed by an experienced surgeon had a potency rate of 29% at the end of 1 year [23]. Ogaya et al. did a similar study wherein they had lesser mean time to return to potency in patients with dHAM versus without dHAM usage, also the potency rates at 1, 3, 6, 9 months were significantly better in dHAM patients at all points of time [8]. In the current study, the potency was higher at all intervals of time in INT versus CON (Table 4). Most patients became potent in the first 3 months following surgery in both groups, and the number of patients was also higher in INT ($p < 0.05$).

In agreement with prior research, age turned out to be the most significant predictor for return of potency even in patients with a high SHIM score preoperatively [24]. So, younger patients stand to gain more with the usage of dHAM.

Cook et al. presented in vitro data showing that a dehydrated membrane (like the one used in the current study) had inferior integrity of the signaling molecules as compared to the cryopreserved membrane [25]. Further in vivo investigations are warranted to determine whether the use of cryopreserved membranes as opposed to dehydrated products is better for potency outcomes.

Chitosan, which is a natural polysaccharide when utilized for the same purpose, was related to significantly improved potency results in a study reported by Francesco Porpiglia et al. [16]. They reported data at 6 months and not 1 year, after using chitosan. The small duration of follow-up and lack of a control arm in their study can be taken as a handicap.

Conclusion

This study supports and reaffirms the use of dHAM for earlier and overall higher probability of satisfactory potency at 1 year after RALP. The enhanced financial impact on the health care system because of dHAM usage needs to be explored and feasibility for use in every patient needs to be examined. This important aspect was not investigated in our study. The earlier and higher possibility of recovering sexual potency may justifiably mitigate the cost of this added procedure of using dHAM in RALP. This is because the long-term expenses incurred with prolonged use of PDE5 inhibitors, vacuum devices, intra penile injections, etc., are avoided.

Limitations

Our study was not completely devoid of limitations. Recall bias during telephonic interviews may have occurred. This being a retrospective non-blinded study some amount of placebo effect might have contributed to the final potency levels of patients who had the membrane wrapped on their nerves. A randomized prospective blinded study with different operators would eliminate the bias emanating out of a single surgeon, the same nerve-sparing technique, and single center study.

Compliance with ethical standards

Financial interest Authors Rajesh Raj Bajpai, Shirin Razdan, Marcos A Sanchez and Sanjay Razdan declare that they have no financial disclosure to make.

Conflict of interest Authors Rajesh Raj Bajpai, Shirin Razdan, Marcos A Sanchez and Sanjay Razdan declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all participating individuals in this study.

Ethical approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration ns the later amendments or comparable ethical standards.

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