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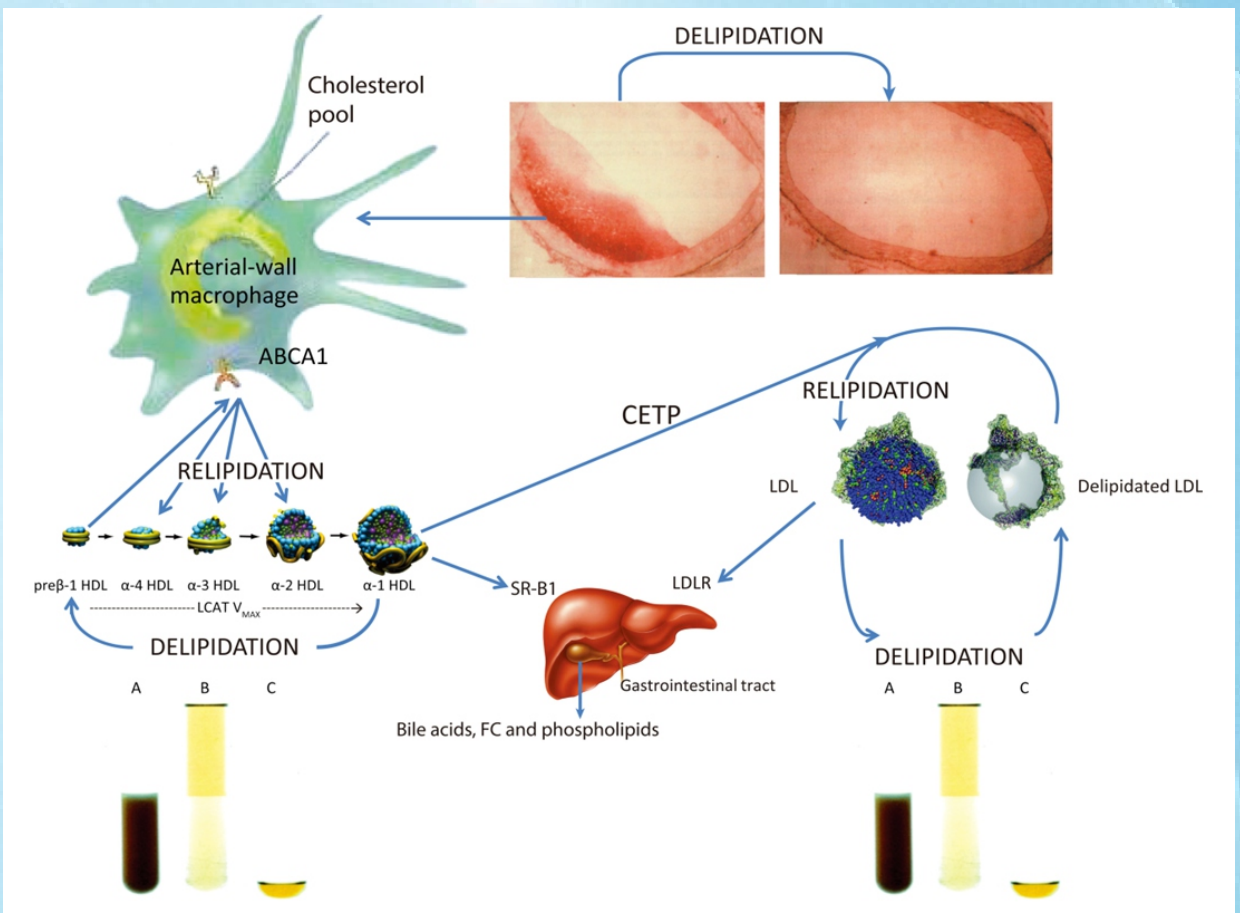

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Transcatheter Closure of Multiple Defects of the Atrial Septum: Technique and Follow-Up

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Abstract

Introduction: Although multiple defects of the atrial septum are not uncommon, there remain limited data regarding the use of multiple devices in these patients. A variety of approaches to transcatheter closure have been used, and in this paper we describe the experience from two operators in a single centre. Methods: From September 2002 to September 2012, 673 transcatheter atrial septal defects (ASD) and patent foramen ovale (PFO) closure procedures were performed and retrospectively examined in a registry analysis. Results: Of these, 22 patients had multiple discrete defects, and four different approaches to closure were used. In 4 patients (18.2%) one device was used percutaneously to close multiple defects. Eleven patients (50%) had two devices inserted during the same procedure while two patients (9%) had two devices inserted as staged procedures. One patient (4.5%) had three devices inserted over two procedures. Four patients (18.2%) were found not to be suitable for percutaneous closure during the procedure and were referred for surgical closure. Conclusion: Our experience with the implant procedures, and clinical follow up of patients, shows that patients with multiple defects can be effectively treated with transcatheter device techniques including single device closure, multiple devices in one procedure and multiple devices in staged procedures and also with surgical repair.

Keywords

Transcatheter, Atrial Septal Defects

1. Introduction

Atrial septal defects (ASD) and patent foramen ovale (PFO) are common cardiac defects. Over the past fifteen

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years, transcatheter device closure has become the more common method of treatment. Transcatheter device occlusion of atrial septal defects has been shown to be as successful as surgical closure and is the preferred option which is feasible and safe given the low incidence of major complications and shorter length of hospital stay [1]-[4]. Closing multiple defects with multiple devices is feasible and has been demonstrated with relative safety and efficacy in both adults and the paediatric population [5] [6].

For single defects, there is a 98% closure rate with no significant residual shunt at follow-up in suitable patients [7] [8]. There are limited data on transcatheter closure of multiple defects of the atrial septum. There is considerable morphological variation in size and location of the defects, and as such there are a variety of approaches to percutaneous closure. One device can be used for multiple defects or alternatively two or more devices can be used. If more than one device is used, these can be inserted during the same procedure or as staged prodecures. A recent study has suggested that there is an increased risk of residual shunt with the use of more than one device for multiple defects [9].

The objectives of our study were to analyse how many patients in our centre with atrial septal defects had multiple lesions, to see which methods of closure were utilised and to follow up these patients to look at their outcomes.

2. Methods

From September 2002 to September 2012, 673 percutaneous ASD and PFO closure procedures were performed and retrospectively examined in a registry analysis. Of these consecutive patients, 22 had multiple septal defects and they were analysed further to determine the method of closure used. There were no inclusion or exclusion criteria. The principal indication for ASD closure was to treat symptoms of dyspnoea, while cryptogenic stroke in the absence of other clear risk factors for stroke was the principal indication for PFO closure. Rarer indications for the closure of PFOs include recurrent transient ischaemic attacks (TIAs), severe migraine refractory to drug therapy and as a prophylactic treatment for decompression illness in professional divers. All patients had a degree of right ventricular impairment.

3. Results

The mean age of the patients was (41.2 ± 9.5), and (78%) were female. The devices used for closure included, the Amplatzer (AGA Medical) Septal occluder (ASO), Amplatzer PFO occluder, the Amplatzer Cribriform device, the Premere device, the Gore Helex occluder, the Gore Septal Occluder, Occlutech devices and CardioSEAL-STARFlex devices. Multiple ASDs or a combination of ASD and PFO were present in 3.3% (n = 22) of all patients in whom closure was attempted. For these patients, a total of 34 closure devices were used of which 22 were Amplatzer PFO and Septal occluders, 2 Helex occluders, 3 Premere devices and 7 Amplatzer Cribriform devices.

Of the 22 patients with multiple defects, four different strategies to closure were used. In 4 patients (18.2%) one device was used percutaneously to close multiple defects. Eleven patients (50%) had two devices inserted during the same procedure while two patients (9%) had two devices inserted as staged procedures over a period of time. One patient (4.5%) had three devices inserted on two separate procedures for three defects. Four patients (18.2%) were found not to be anatomically suitable for percutaneous closure during the procedure and went on to be referred for surgical closure. Examples of cases from each of these four groups are described below (**Table 1**).

We have obtained long term follow up data for 15 of the 22 patients with multiple defects. The follow up was conducted in September 2014, between 2 and 12 years from the original procedures. Of the 15 patients on whom

Table 1. Four strategies for multiple defect closure.

	Number of patients	% of total (n = 22)
One Device	4	18
Multiple Devices—Same procedure	11	50
Multiple Devices—Staged procedure	3	14
Surgical closure	4	18

long term follow up data is available, two patients have died, one of whom had multiple medical comorbidities. One patient has developed palpitations and has a small device leak seen on TOE which is being medically managed. Another patient developed atrial fibrillation and a pulmonary embolus post-procedure. The remaining patients were well at follow up. Of the patients referred for surgery follow up data was obtained for 2 of the 4 patients and they are well.

3.1. Single Device Closure

Case One: A 27-year-old lady with a history of cryptogenic stroke was found on echocardiography to have a PFO and also a small ASD. A 35 mm Cribriform Atrial Septal Occluder was placed across the ASD which closed both holes with a good result. Post-procedure transthoracic echocardiography (TTE) showed a minute flash within the closure device on colour flow Doppler. Subsequently she experienced a transient episode of pins and needles in her left arm and leg, although CT head demonstrated no abnormality. Repeat echocardiography at 5 weeks confirmed the device was well seated with no residual shunt.

3.2. Multiple Devices, One Procedure

Case Two: A 52-year-old man was found to have an atrial septal aneurysm on echocardiography and a positive bubble transoesophageal echocardiogram (TOE), following a stroke. Two devices were implanted, one across the PFO and one across an adjacent ASD. Post-procedure TTE showed no residual shunt. Repeat echocardiography at 4 months showed minimal left to right colour flow. Post closure of the defect, the patient developed atrial fibrillation (AF).

Case Three: A 37-year-old lady was found on TOE to have two atrial septal defects—one at the inferior border of the septum and one large defect superiorly, which was multifenestrated. The upper defect was closed first and the lower defect reassessed. There was ongoing flow through the smaller defect, and so a second device was deployed across it (see **Figure 1**). The devices were aligned at right angles to one another because of the aneurysmal nature of the septum. There was some residual flow through the larger device, however an echocardiogram 15 months later showed no obvious residual shunt. The patient developed post-procedure haematemesis and oesophagogastroduodenoscopy (OGD) showed a Mallory-Weiss tear. Therefore Clopidogrel was given in place of Aspirin.

Case Four: A 72-year-old lady was admitted for elective closure of ASD. After the 26 mm ASO was placed, it became clear that there was a second defect. A further 12 mm ASO was used and in order to interdigitate the devices correctly, the discs were retrieved and then redeployed in the following order: left atrium (LA) disc of smaller device, LA disc of larger device, right atrium (RA) disc of larger device, RA disc of smaller device (see

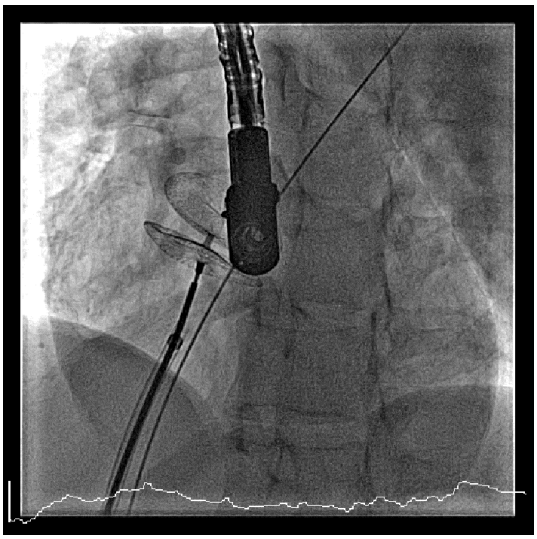

Figure 1. Amplatzer septal occluder device in place with 0.035 guidewire across second defect.

Figure 2 and **Figure 3**). There was no residual shunt and follow up TTE at 4 months showed some residual flow with a small left to right shunt.

3.3. Multiple Devices, Staged Procedure

Case Five: A 36-year-old man was admitted for PFO closure post-cerebrovascular event. During the procedure when the interatrial septal anatomy was studied, it was found that there was both an ASD and a PFO. A single device was deployed across the ASD (ASO) which was thought to also occlude the PFO. However, 7 months later the patient required a further procedure for closure of the PFO due to ongoing residual shunt using an Amplatzer PFO occluder. Post-procedure TTE showed resolution of the shunt.

3.4. Unsuccessful Percutaneous Closure (Surgical Referral)

Case Six: A 49-year-old lady was admitted electively for ASD closure. TOE showed multiple defects in the atrial septum. A device was placed in an attempt to close the septal defects, but after deployment there were still

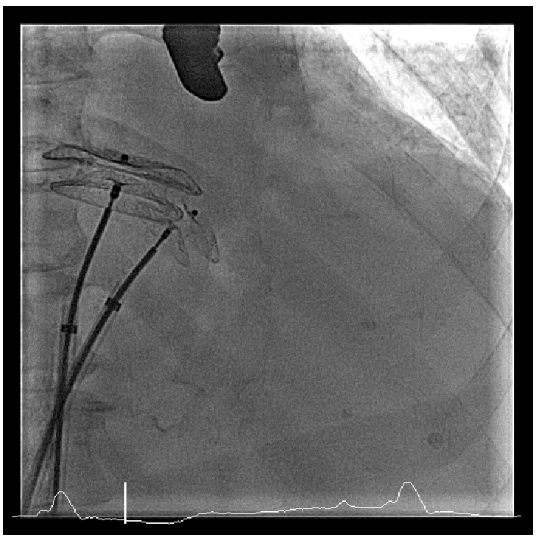

Figure 2. Simultaneous deployment of two ASO devices, interdigitating.

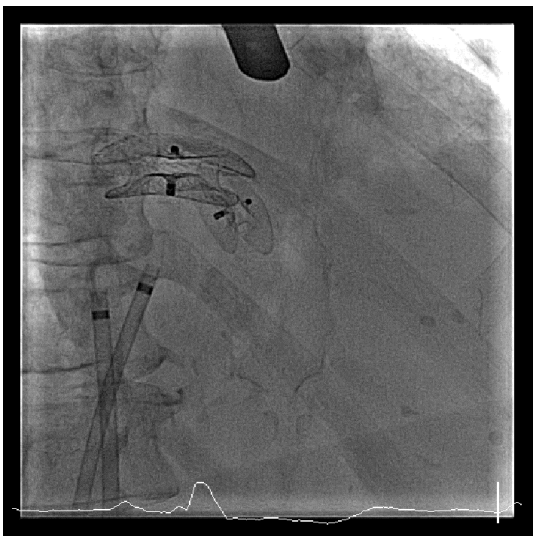

Figure 3. Devices after deployment.

multiple shunts at both ends of the device. The device was therefore removed and the patient was referred for surgical closure.

4. Discussion

Limited data exist on the outcome of using multiple devices to close multiple defects of the atrial septum. A single device is generally used if it can effectively close two adjacent defects. It is deployed across the largest defect and ideally covers any adjacent defects. Multiple devices are more commonly used when the separation of the defects is more than a few millimetres.

Single device occlusion is more cost effective, avoids a bulky profile and results in shorter fluoroscopy times. If two or more devices are used they must be placed either simultaneously (with one sandwiching the other) or sequentially, either at the same procedure or in a staged procedure sequence. The interleaving technique had been shown to achieve the lowest possible profile with overlapping devices (see **Figure 4**).

This method will reduce the risk of device embolisation and also decreases pressure on surrounding structures. As it is not possible to interleave at a later stage, a staged procedure using multiple devices leads to overlapping and a higher profile [10]-[12]. However, because most residual defects close over a period of time it has been suggested that a single device be used initially and then a second device at a later stage if necessary, which may result in fewer and smaller second devices [13].

Device erosion is a recognised complication of transcatheter ASD closure. Device diameter, multiple devices, deficient rims in vulnerable areas (e.g. antero-superior rims) all increase the risk of erosion. Of cases reported, very few have involved structural deterioration of the actual devices but in comparison atrial free wall erosion is the more common complication. Furthermore it does not appear that more than one device is a risk factor for erosion, but this may simply reflect the paucity of cases undertaken.

Imaging techniques including transesophageal (TOE) echocardiography are used to measure the extent of tissue between multiple defects and ideally at least 6 - 7 mm is needed in order for two devices to be used [14]. However, even with a separation of more than 7 mm, it may still be possible to use a single device depending upon the precise location and morphology of the defects.

There are no differences between the single device and the multiple devices groups in terms of anti-platelet therapy and endocarditis prophylaxis and treatment.

5. Conclusion

Our experience shows that our patients with multiple defects were treated with one of four methods—single device closure, multiple devices in one procedure, multiple devices in staged procedures and unsuccessful percutaneous repair requiring surgical referral. These registry data confirm that transcatheter closure for multiple defects appears to be safe and efficiacious in selected cases but the study is limited by small numbers. Although

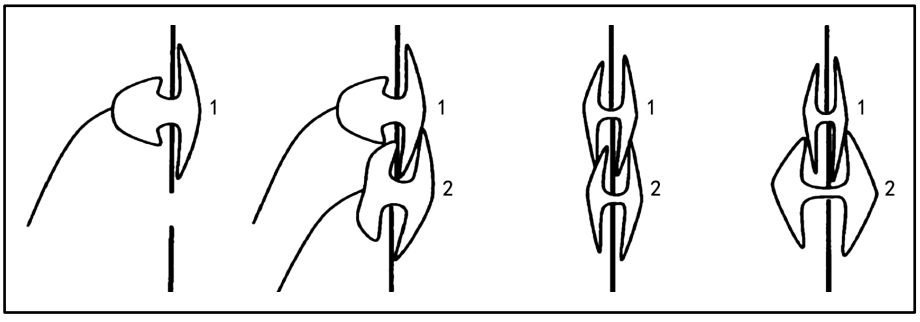

Figure 4. Technique of “interleaving”. (a) Deployment of device I, maintaining tension on right atrial disk with delivery wire; (b) Placement of device 2 so that left atrial disk lies on left atrial side of device 1. While maintaining tension on device 1, the right atrial disk of device 2 is deployed; (c) Release of tension on device 1 allows its right atrial disk to rest on right atrial side of device 2. The result is the lowest possible profile; (d) Overlapping devices without “interleaving” and a resulting high profile of device 2 [8].

follow-up data are incomplete, the results are encouraging and there is no obvious difference in outcomes between the four groups.

References

[1] Teoh, K., Wilton, E., Brecker, S. and Jahangiri, M. (2006) Simultaneous Removal of an Amplatzer Device from an Atrial Septal Defect and the Descending Aorta. *The Journal of Thoracic and Cardiovascular Surgery*, **131**, 909-910.

[2] Butera, G., *et al*. (2006) Percutaneous versus Surgical Closure of Secundum Atrial Septal Defect: Comparison of Early Results and Complications. *American Heart Journal*, **151**, 228-234. http://dx.doi.org/10.1016/j.ahj.2005.02.051

[3] Butera, G., *et al*. (2010) Percutaneous Closure of Multiple Defects of the Atrial Septum: Procedural Results and Long-Term Follow-Up. *Catheterization and Cardiovascular Interventions*, **76**, 121-128. http://dx.doi.org/10.1002/ccd.22435

[4] Tillman, T., Mulingtapang, R. and Sullebarger, J.T. (2008) Approach to Percutaneous Closure in Patients with Multiple Atrial Septal Defects. *Journal of Invasive Cardiology*, **20**, E167-E170.

[5] Mahadevan, V.S., *et al.* (2009) Transcatheter Closure of Atrial Septal Defects with Multiple Devices in Adults: Procedural and Clinical Outcomes. *International Journal of Cardiology*, **133**, 359-363. http://dx.doi.org/10.1016/j.ijcard.2008.01.027

[6] Bramlet, M.T. and Hoyer, M.H. (2008) Single Pediatric Center Experience with Multiple Device Implantation for Complex Secundum Atrial Septal Defects. *Catheterization and Cardiovascular Interventions*, **72**, 531-537. http://dx.doi.org/10.1002/ccd.21668

[7] D Roy, R.S., Bunce, N., Ward, D. and Brecker, S.J. (2012) Selecting the Optimal Closure Device in Patients with Atrial Septal Defects and Patent Foramen Ovale. *Interventional Cardiology*, **4**, 85-100. http://dx.doi.org/10.2217/ica.11.90

[8] Roman, K.S., *et al.* (2002) Different Techniques for Closure of Multiple Interatrial Communications with the Amplatzer Septal Occluder. *Journal of Interventional Cardiology*, **15**, 393-397. http://dx.doi.org/10.1111/j.1540-8183.2002.tb01073.x

[9] Hu, Z., *et al*. (2015) Comparison of the Effectiveness and Safety of Single versus Dual Occluders for the Closure of Multiple Atrial Septal Defects. *Journal of Interventional Cardiology*, **27**, E90-E97.

[10] Cao, Q., *et al*. (2000) Transcatheter Closure of Multiple Atrial Septal Defects. Initial Results and Value of Two- and Three-Dimensional Transoesophageal Echocardiography. *European Heart Journal*, **21**, 941-947. http://dx.doi.org/10.1053/euhj.1999.1909

[11] Szkutnik, M., *et al*. (2004) Transcatheter Closure of Double Atrial Septal Defects with a Single Amplatzer Device. *Catheterization and Cardiovascular Interventions*, **61**, 237-41. http://dx.doi.org/10.1002/ccd.10753

[12] Masseli, J., *et al*. (2013) Transcatheter Closure of Multiple Interatrial Communications. *Catheterization and Cardiovascular Interventions*, **81**, 825-836.

[13] Song, Z.-Y., *et al*. (2009) Clinical Efficiency and Safety Analysis of Transcatheter Closure of Multiple Atrial Septal Defects in Adults. *Clinical Cardiology*, **32**, 130-134. http://dx.doi.org/10.1002/clc.20450

[14] Kuo, B.T., *et al.* (2011) Double Atrial Septal Defect: Diagnosis and Closure Guidance with 3D Transesophageal Echocardi-Ography. *Echocardiography*, **28**, E115-E117. http://dx.doi.org/10.1111/j.1540-8175.2010.01347.x

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Pain and Radiographic Outcomes in Adult Idiopathic Scoliosis Patients Using a Scoliosis Activity Suit: An 18-Month Case Controlled Chart Review

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Abstract

There are few conservative treatment options for patients with adult idiopathic scoliosis. These typically include pharmacologic pain management, epidural injections, and generalized CAM treatments such as massage and chiropractic manipulation. The purpose of this study was to compare the post-treatment results in patients wearing the scoliosis activity suit versus baseline assessments as well as adult scoliosis patients who did not wear the activity suit. The pain and Cobb angle outcomes of 53 consecutive patients with adult idiopathic scoliosis following a trial of a scoliosis activity suit were reviewed. The average scores and measurements at 18 months were statistically significantly improved for both the quadruple numerical pain rating scale as well as Cobb angle. The scoliosis activity suit may be a viable supportive therapy for the treatment of chronic pain associated with adult idiopathic scoliosis. Further prospective studies should evaluate treatment effects of this suit using intent-to-treat methodology.

Keywords

Scoliosis, Pain, Spine, Rehabilitation

[*]Corresponding author.

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1. Introduction

The treatment options for idiopathic scoliosis have greatly developed over the last decade. Bracing techniques [1] and surgical techniques [2] have consistently been made available to adolescent idiopathic scoliosis patients. However, many of the non-surgical procedures have not been available to adult idiopathic scoliosis patients. It is well know that idiopathic scoliosis can continue to progress even into adulthood [3]. When an idiopathic scoliosis measures >30˚ at the time of skeletal maturity, there is approximately a 68% chance that the curve will continue to progress thereafter [4] [5]. For example, a study by Marty-Poumarat *et al.* [6], reported a near linear rate of progression year by year into later adulthood.

Most of the treatment studies in adults with idiopathic scoliosis have been surgical. However, common among the various surgical techniques is the incidence of perioperative and postoperative complications, ranging from 17% - 55% [7]-[9]. The chance of revision surgery in a study of 306 patients was 44% in the first 6 years [10]. Since rigid scoliosis bracing is typically indicated for skeletally immature patients, rigid bracing in adulthood is not considered necessary [11]. According to the Society on Scoliosis Orthopedic and Rehabilitation Treatment (SOSORT), bracing for adult curves is indicated only when the therapeutic value of the brace chosen has been established [12]. However, many of the brace technologies established for adult scoliosis patients are European, and their availability in the US is limited at the present time.

With the potential risks of surgical intervention in adulthood, and the limited availability of European bracing technologies in the United States, we report the results of a scoliosis activity suit used in a cohort of patients with adult idiopathic scoliosis.

Previous early data suggest [13] that this suit may help certain pain and radiographic parameters of scoliosis. Here we present our data against a previously published control group with established progression data in adult scoliosis cases.

The scoliosis activity suit is a neoprene, rotation-based activity suit, composed of 4 distinct pieces. The novelty of the scoliosis activity suit is that it creates a rotational resistance into the torso that accentuates the rotational displacement of the scoliosis. This increased rotational stimulus is thought to elicit a corrective postural reflex that results in de-rotation of the spine out of the scoliosis curvature.

2. Intervention and Outcomes

Patient files from a single multidisciplinary medical center were consecutively pulled for analysis. All patients had previously reported to this clinic with an established history of adult idiopathic scoliosis. Inclusion criteria for this study included 1) Prior to treatment intervention, all patients completed a quadruple numerical pain rating scale, 2) patients were all skeletally mature at baseline, 3) baseline Cobb angle of at least 30˚, and 4) they were not receiving concurrent treatment or being followed up with elsewhere. Patient files were excluded if the type of scoliosis was not idiopathic, as well as patients with history of scoliosis fusion surgery. A total of 53 patient files were consecutively selected, so as to minimize selection bias.

Patients had participated in a fitting process, where the focus was on teaching each patient to properly fit and adjust his/her scoliosis activity suit based upon his/her respective curve pattern. They were also given instruction on which specific activities of daily living to perform while wearing the activity suit. Patients were instructed to wear the activity suit for a total of 4 - 6 hours per day total, in two separate sessions (2 - 3 hours each ideally). **Figure 1** shows an illustration of a typical configuration for an adult patient.

Once the patients completed their fitting process, they were instructed to report back every 3 months for 1 year, followed by 6-month intervals thereafter. At each 6-month interval, patients again completed the numerical pain rating scale. These questionnaires were compared to baseline to determine their pain and changes.

The gender breakdown among the 53 total patients selected for this study was 41 females and 12 males. Of these 38 had curves above 30˚ while 15 had curves above 50˚. When categorized based upon curve pattern, there were 8 patients with thoracolumbar curves, 19 with thoracic curves, 10 with lumbar curves, and 16 with double major curves.

Statistical analysis was performed for the curve pattern groups as well as the baseline Cobb angle groups. Student t-tests were performed to evaluate before and after treatment comparisons. **Tables 1-5** provide an outline of this data for the outcome assessments. Across the entire cohort, 79% of patients achieved a curve correction of 6˚ or greater, 17% had curves unchanged or stabilized (defined as <6˚ of change), and 4% saw their curves increase. When subdividing the entire cohort by curve pattern, the thoracolumbar group achieved the

Figure 1. A common sample configuration of the scoliosis activity suit.

Table 1. Entire cohort.

Patient	Gender	CurveType	Cobb1	Cobb2	QVAS1	QVAS2
1	f	L	55	50	63	43
2	m	D	32	22	47	37
3	f	L	36	26	60	53
4	m	T	52	33	43	33
5	f	TL	34	30	70	43
6	f	T	42	29	40	20
7	f	T	44	26	30	20
8	f	L	66	40	73	47
9	m	TL	47	30	53	43
10	f	D	33	20	33	30
11	f	TL	38	30	60	37
12	m	T	52	35	73	47
13	f	L	61	51	63	47
14	f	T	35	30	33	20
15	f	D	49	36	37	20
16	f	D	39	33	43	10
17	f	L	40	33	57	30
18	f	D	46	29	43	27
19	m	T	56	28	57	43
20	f	D	31	20	47	40
21	f	T	38	25	43	30
22	f	L	38	24	67	40

Continued

23	f	D	33	20	37	13
24	m	D	32	26	43	27
25	m	T	30	21	20	20
26	f	D	38	27	33	17
27	f	D	47	33	43	27
28	f	T	41	35	37	30
29	f	L	65	55	60	27
30	f	T	40	18	33	17
31	f	T	54	26	57	27
32	m	TL	39	31	67	23
33	f	T	34	34	43	40
34	f	D	58	59	43	43
35	f	T	37	29	47	40
36	f	TL	39	20	77	63
37	f	L	53	49	53	43
38	f	T	44	40	53	47
39	f	D	46	48	60	23
40	f	T	61	56	73	33
41	m	D	45	42	43	40
42	f	L	43	39	50	40
43	m	T	60	53	60	47
44	f	TL	48	37	50	37
45	f	T	32	30	37	30
46	f	D	59	52	43	37
47	f	L	39	31	57	33
48	m	T	53	46	37	20
49	f	TL	31	15	53	37
50	f	D	30	26	47	30
51	f	D	34	21	53	47
52	f	TL	32	23	47	40
53	m	T	62	50	67	27
	P value			1.36204E−06		1.03956E−10
	Mean		43.830189	33.43396226	50.150943	33.67924528
	42	79%	>6˚			
	9	17%	<6˚			
	2	4%	Progressed			

Table 2. Lumbar curves.

Patient	Gender	CurveType	Cobb1	Cobb2	QVAS1	QVAS2
1	f	L	55	50	63	43
3	f	L	36	26	60	53
8	f	L	66	40	73	47
13	f	L	61	51	63	47
17	f	L	40	33	57	30
22	f	L	38	24	67	40
29	f	L	65	55	60	27
37	f	L	53	49	53	43
42	f	L	43	39	50	40
47	f	L	39	31	57	33
		P value		0.035705		6.80694E−06
		Mean	49.6	39.8	60.3	40.3
	7	70%				
	3	30%				
	0					

Table 3. Double curves.

Patient	Gender	CurveType	Cobb1	Cobb2	QVAS1	QVAS2
23	f	D	33	20	37	13
24	m	D	32	26	43	27
26	f	D	38	27	33	17
27	f	D	47	33	43	27
34	f	D	58	59	43	43
39	f	D	46	48	60	23
41	m	D	45	42	43	40
46	f	D	59	52	43	37
50	f	D	30	26	47	30
51	f	D	34	21	53	47
		P value		0.01719916		8.47507E−05
		Mean	40.75	32.125	43.4375	29.25
	12	75%				
	2	12.50%				
	2	12.50%				

Table 4. Thoracic curves.

Patient	Gender	CurveType	Cobb1	Cobb2	QVAS1	QVAS2
4	m	T	52	33	43	33
6	f	T	42	29	40	20
7	f	T	44	26	30	20
12	m	T	52	35	73	47
14	f	T	35	30	33	20
19	m	T	56	28	57	43
21	f	T	38	25	43	30
25	m	T	30	21	20	20
28	f	T	41	35	37	30
30	f	T	40	18	33	17
31	f	T	54	26	57	27
33	f	T	34	34	43	40
35	f	T	37	29	47	40
38	f	T	44	40	53	47
40	f	T	61	56	73	33
43	m	T	60	53	60	47
45	f	T	32	30	37	30
48	m	T	53	46	37	20
53	m	T	62	50	67	27
		P Value		0.00071025		0.0003936
		Mean	45.6315789	33.8947368	46.4736842	31.1052632
	16	84%				
	3	16%				

Table 5. Thoracolumbar curves.

Patient	Gender	CurveType	Cobb1	Cobb2	QVAS1	QVAS2
5	f	TL	34	30	70	43
9	m	TL	47	30	53	43
11	f	TL	38	30	60	37
32	m	TL	39	31	67	23
36	f	TL	39	20	77	63
44	f	TL	48	37	50	37
49	f	TL	31	15	53	37
52	f	TL	32	23	47	40
		P value		0.002114		0.00167
		Mean	38.5	27	59.625	40.375
	7	88%				
	1	12%				

largest correction, with 88% of thoracolumbar curves correcting ≥6˚. The thoracic group achieved this in 84% of patients. By contrast, the lumbar group saw the lowest correction rate at 70%, followed by the double major group at 75%. However, the double major group is the only group in which patients saw their curves worsen at follow-up (12.5%). The curve progression in each patient was 1˚ and 2˚ respectively. This is approximately equal to the natural history of progression for these curves. **Figure 2** shows some of the postural changes triggered by application of the suit.

When comparing these curve patterns to the published control group by Marty-Poumarat *et al.* [6], the average initial age of their subjects was 37 years; ours was 44. The average starting Cobb angle for lumbar and thoracolumbar curves was 30˚, while our lumbar group started at 50° and our thoracolumbar group was 39˚. Thoracic curves began at 44˚ for the control subjects, and our thoracic group began with 46˚ curves. According to Marty-Poumarat *et al.*'s data [6], they observed average thoracic curve progression of 0.68˚ per year. This would equate to roughly 1.02˚ curve increase over our present 18-month study timeframe. Our thoracic group saw their curves improve, by contrast, of 12˚ ($P < 0.05$). Lumbar and thoracolumbar curves increased 1.45˚ per year in the control group, whereas our lumbar group saw an average curve correction of 10˚ ($P < 0.05$) and the thoracolumbar group corrected an average 11˚ ($P < 0.05$).

3. Discussion

Since most adult scoliosis patients seek therapy for their scoliosis due to pain [12], it is logical to evaluate those methods that seek to accomplish this goal, even if curve correction is unattainable.

Our control group was composed of a previously published cohort by Marty-Poumarat *et al.* [6]. Our present cohort is similar in demographics and curve characteristics to their cohort, which is why it was chosen. Since Marty-Poumarat *et al*. provided a detailed, linear rate of progression for adult scoliosis patients, it gave us a realistic comparative by which to evaluate if the scoliosis activity suit could potentially alter the course of natural history of this disease process.

It is important to discuss the limitations of this study. While we did attempt to control the study, it was retrospective in nature, which invariably provides for some degree of selection bias, although we attempted to minimize this by selecting our inclusion criteria before reviewing and selecting patient charts. We also did not perform an intent-to-treat analysis, which would have accounted for the subjects who did not report for follow-up at 18 months. Future studies should include this analysis.

Although this treatment was primarily performed at home, out present study does not take into account the percentage of compliance within our study population. It is possible that our outcomes could be correlated to the rate of compliance, as well as for those we lost to 18-month follow-up.

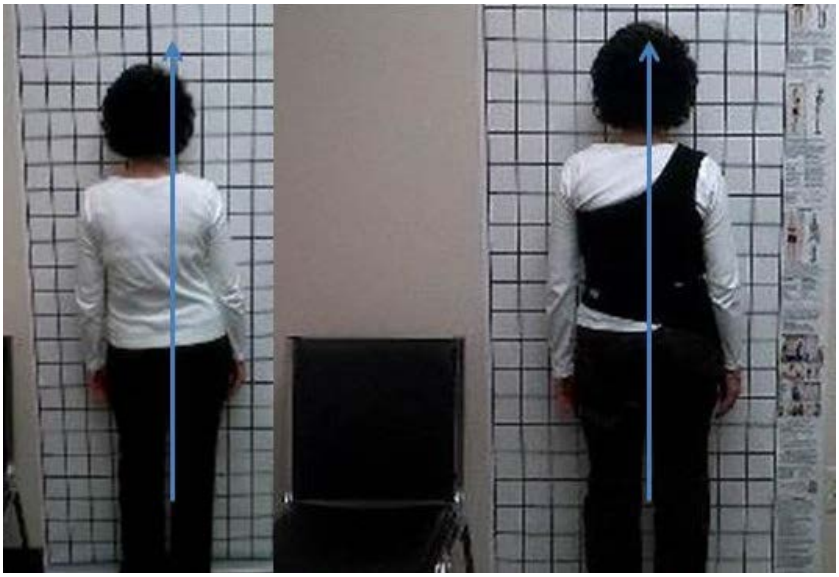

Figure 2. Postural changes associated with the scoliosis activity suit.

Finally, while pain is a reasonable outcome assessment for this patient population in particular, the quadruple visual analog scale was not specifically designed for scoliosis patients. However, it does provide an easy-to-collect dataset from which to evaluate treatment impact on pain levels. Future studies should include quality of life indices specifically designed for the scoliosis patient population.

Acknowledgements

The authors declare none.

Conflicts of Interest

MWM has applied for design and utility patents in the USA on the scoliosis activity suit. The remaining authors declare no conflicts of interest.

References

[1] Weinstein, S.L., Dolan, L.A., Wright, J.G. and Dobbs, M.B. (2013) Effects of Bracing in Adolescents with Idiopathic Scoliosis. *The New England Journal of Medicine*, **369**, 1512-1521. http://dx.doi.org/10.1056/NEJMoa1307337

[2] Janicki, J.A. and Alman, B. (2007) Scoliosis: Review of Diagnosis and Treatment. *Paediatrics & Child Health*, **12**, 771-776.

[3] Collis, D.K. and Ponseti, I.V. (1969) Long-Term Follow-Up of Patients with Idiopathic Scoliosis Not Treated Surgically. *The Journal of Bone & Joint Surgery* (*Am*), **51**, 425-445.

[4] Weinstein, S.L., Dolan, L.A., Spratt, K.F., Peterson, K.K., Spoonamore, M.J. and Ponseti, I.V. (2003) Health and Function of Patients with Untreated Idiopathic Scoliosis: A 50-Year Natural History Study. *JAMA*, **289**, 559-567. http://dx.doi.org/10.1001/jama.289.5.559

[5] Weinstein, S.L. (1999) Natural history. *Spine*, **24**, 2592-2600. http://dx.doi.org/10.1097/00007632-199912150-00006

[6] Marty-Poumarat, C., Scattin, L., Marpeau, M., Garreau de Loubresse, C. and Aegerter, P. (2007) Natural History of Progressive Adult Scoliosis. *Spine*, **32**, 1227-1234. http://dx.doi.org/10.1097/01.brs.0000263328.89135.a6

[7] Yadla, S., Maltenfort, M.G., Ratliff, J.K. and Harrop, J.S. (2010) Adult Scoliosis Surgery Outcomes: A Systematic Review. *Neurosurgical Focus*, **28**, E3. http://dx.doi.org/10.3171/2009.12.FOCUS09254

[8] Li, G., Passias, P., Kozanek, M., Fu, E., Wang, S., Xia, Q., *et al*. (2009) Adult Scoliosis in Patients over Sixty-Five Years of Age. Outcomes of Operative versus Nonoperative Treatment at a Minimum Two-Year Follow-Up. *Spine*, **34**, 2165-2170. http://dx.doi.org/10.1097/BRS.0b013e3181b3ff0c

[9] Brodano, B.G., Terzi, S., Gasbarrini, A., Bandiera, S., Simoes, C. and Boriani, S. (2013) Do Benefits Overcome the Risks Related to Surgery for Adult Scoliosis? A Detailed Analysis of a Consecutive Case Series. *European Spine Journal*, **22**, S795-S802. http://dx.doi.org/10.1007/s00586-013-3031-y

[10] Charosky, S., Guigui, P., Blamoutier, A., Roussouly, P. and Chopin, D. (2012) Study Group on Scoliosis. Complications and Risk Factors of Primary Adult Scoliosis Surgery. A Multicenter Study of 306 Patients. *Spine*, **37**, 693-700. http://dx.doi.org/10.1097/BRS.0b013e31822ff5c1

[11] Scoliosis Research Society. https://www.srs.org/patient_and_family/scoliosis/idiopathic/adolescents/bracing.htm

[12] Negrini, S., Aulisa, A.G., Aulisa, L., Circo, A.B., de Mauroy, J.C., Durmala, J., *et al*. (2012) 2011 SOSORT Guidelines: Orthopaedic and Rehabilitation Treatment of Idiopathic Scoliosis during Growth. *Scoliosis*, **7**, 3. http://dx.doi.org/10.1186/1748-7161-7-3

[13] Morningstar, M. (2013) Outcome Observations in Patients Using a Scoliosis Activity Suit: A Retrospective Chart Review after One-Year Follow-Up. *Journal of Scoliosis Rehabilitation*, **10**, 1-10.

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Patient Adherence to a Cardiovascular Rehabilitation Program: What Factors Are Involved?

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Abstract

Background: Phase II cardiac rehabilitation (CR) is a class IA indication in patients suffering a cardiovascular event (CV). Current guidelines suggest 36 exercise sessions over a period of 3 months. The main aim of this study was to analyze the rate of adherence to a cardiac rehabilitation program and the factors influencing it. Methods: This was a cross-sectional study in 421 secondary prevention patients, who assisted to a Phase-II-CR program between 2007 and 2014. At baseline and program end, patients completed a 6-minute walk test and the Short-Form 36 Health Survey (SF-36). Vital signs and anthropometric measurements were also collected. Adherence was quantified as the percentage of individuals who attended all 36 sessions of the program. Factors considered for affecting adherence included: cardiovascular risk factors (RFs), type of health insurance (public or private), aerobic capacity, and SF-36 score parameters. Results: Adherence to Phase-II-CR was 33%, with no significant differences between men and women. The regression model fully adjusted for age, sex, RFs, type of health insurance and SF-36 score, showed that a SF-36 score <50 on physical health (odds ratio (OR): 11.47; 3.99 - 32.99; $p < 0.0001$) and smoking (OR: 4.41; 1.25 - 15.62; $p = 0.02$) were strong predictors for non-adherence. A trend for better adherence was observed in subjects older than 50 years compared to those aged between 17 and 50 years (37% versus 23%, respectively; $p = 0.05$). No significant differences were observed in adherence according to RFs clustering. Conclusions: Adherence to Phase-II-CR is low in our population. Patient-related factors, such as SF-36 score and smoking, were the best determinants of Phase-II-CR adherence. Health system-related factors did not influence adherence in this population. Prospective

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studies are warranted to determine all the factors which may influence adherence to Phase-II-CR programs.

Keywords

Cardiac Rehabilitation, Adherence, Secondary Prevention

1. Introduction

Cardiovascular disease (CVD) is the most common cause of death in Chile and is responsible for 27% of total deaths. Approximately 35% of all CV deaths are due to acute myocardial infarction (MI) [1] and although the rate of MI mortality has remained steady in recent years, its incidence in the younger population has increased significantly [2]. Therefore, it is of utmost importance to promote lifestyle interventions in these patients. Cardiovascular rehabilitation (CR) programs are associated with a reduction in CVD morbidity and mortality as well as improvements in the control of cardiovascular risk factors (RFs), exercise tolerance, control of anxiety and stress, and quality of life, among others [3]-[6].

The latest guidelines from the American College of Cardiology (ACC), the American Heart Association and the European Society of Cardiology have positioned CR programs with an indication class I, level A in secondary prevention [7]. The completion of the 36 sessions (over approximately 3 months) of Phase-II-CR has been associated with a lower risk of death and MI at 4 years compared with those performing fewer sessions [8]. Recent studies have also demonstrated a dose-response relationship in CR (*i.e.*, the greater the adherence to the program, the lower the CVD morbidity and mortality) [8] [9]. Despite these benefits, participation in CR programs remains low, with only 10% to 20% of patients who survive an MI entering a secondary prevention program [8] [10]. This percent is even lower in Latin America with a referral rate around 5% [11]. Moreover, a significant percentage of these individuals leave the program within the first two weeks [12]. The reasons for poor adherence to and underutilization of CR appear to be multifactorial: the patient's age, income, distance from the health center, and personal beliefs of referring physicians about the benefits of Phase-II-CR are among some of the related factors [10] [12]-[14]. Patient specific factors, such as cardiovascular RFs and RF clustering, may also influence adherence to this treatment. Despite the importance of Phase-II-CR as part of the secondary prevention after a CVD event, it is not included as part of hospital discharge instructions for most patients in several countries in Latin America.

Finally, adherence to CR programs is critically important to get the maximum benefits of exercise and the improvement in functional capacity [5] [9]. Therefore, the main aim of this study was to describe adherence rates to a Phase-II-CR program in Chile and evaluate the factors that influence it.

2. Materials and Methods

The study used a cross-sectional design and included a sample of subjects who participated in a Phase-II-CR program between 2007 and 2014 in an ambulatory setting of a university hospital. Exclusion criteria included subjects who did not have any history of atherosclerotic cardiovascular disease. Therefore, the sample consisted of 421 men and women with definite history of coronary heart disease (CHD). Mean age was of 59 ± 12 years.

2.1. Data Collection

Data were collected on the admission to the Phase-II-CR program. All subjects were interviewed by the nurse in charge of the program to obtain demographic, medical cardiovascular RF history, and medication intake data. All the information was entered in a database specifically designed for the program. Upon admission, each subject underwent a graded symptom-limited exercise stress test on a treadmill. Total duration of exercise, metabolic equivalents (METs) and maximum blood pressure and heart rate were recorded. In addition, a 6-minute walk test (6MWT), anthropometric parameters, and the 36-item Short Form Health Survey (SF-36) were measured and collected at baseline and at the end of the 36 sessions. For the 6MWT, the total distance walked and vital signs were recorded.

The following criteria were identified as conditions that could influence patient adherence to the Phase-II-CR program:

1) Hypertension: subjects with physician-stated diagnosis of hypertension, with or without drug treatment.

2) Dyslipidemia: subjects with physician-stated diagnosis of hypercholesterolemia, hypertriglyceridemia and/or low HDL (<40 or <50 mg/dL, in men and women respectively), with or without drug treatment.

3) Obesity: subjects with BMI ≥30 mg/kg^2 and those with abdominal obesity ≥90 cm (men) and ≥80 cm (women) according to published criteria [15] [16].

4) Diabetes and insulin resistance: those with medical diagnosis, with or without medication.

5) Physical inactivity: classification of "sedentary leisure time" was used [17]. Sedentary subjects were defined as those who reported no sport or physical activity of at least 30 minutes, 3 times per week, outside of working hours during the last month. This method has previously shown a direct and significant correlation with maximal aerobic power measured in METs [18].

6) Smoking: categorized by self-report as follows: no smoking, daily smoking (≥1 cigarette/day), current smoking (daily + occasional, <1 cigarette/day), or ex-smoker (last cigarette >6 months) [17].

7) Family history of CHD: present if a subject had a direct relative (male <55 years old or woman <65 years old) who suffered a cardiovascular event.

8) Type of health insurance: Private (ISAPRE) or Public (FONASA) health insurance.

9) Aerobic capacity: total METs achieved in the exercise stress.

10) Quality of life: emotional and Physical health score on SF-36 was used [19].

All subjects admitted to the program provided written informed consent approved by the local ethics committee, which authorized the use of data for academic purposes, protecting confidentiality.

2.2. Adherence

The concept of adherence was applied to the total group of subjects, and it was quantified as the percentage of subjects who attended all 36 sessions of the Phase-II-CR program.

2.3. Short Form 36 (SF-36) Health Survey

The SF-36 consists of 36 questions including domains of physical functioning, physical role, body pain, general health, vitality, social function, emotional role, and mental health. Scores are subdivided into two sub-scales corresponding to two components: physical and emotional health according to the standards of the US SF-36. [19]. This survey was conducted upon the admission of the patient to the Phase-II-CR program and after its completion (conclusion of the 36 sessions). Scores less than 50 for each component were used as cut-offs values of low quality of life.

2.4. Clustering of Risk Factors

Risk-factor clustering was presented as the cumulative number of RFs previously described in the condition's criteria detailed above (from 1 to 7). According to this clustering, subjects were classified into 3 groups: Group 1: 0 to 1 RF, Group 2: 2 to 4 RFs, and Group 3: >4 RFs.

2.5. Statistical Analysis

The results of the tables were expressed as mean (SD) or frequency (%). Comparisons between sex were based on ANOVA and Fisher exact test. Comparisons among adherence SF-36 survey, and baseline functional capacity were determined using Student's t-test.

To analyze the existence of factors which positively or negatively affected adherence, a logistic regression model, adjusted for age, sex, RFs, type of health insurance, and score in the SF-36 health survey was used.

3. Results

A total of 421 subjects were included in this analysis: 18% were women; mean age of the group was 59 ± 12 years old. **Table 1** displays demographic characteristics and risk factor prevalence of the total group according to adherence status (adherent versus non-adherent). The rate of adherence to the 36 sessions of Phase-II-CR

Table 1. Demographic characteristics and risk factor prevalence of the total group according to adherence to the Phase II cardiac rehabilitation program.

Characteristics	Total Group (n = 421)	Adherent Group (n = 141)	Non-adherent Group (n = 280)	*p*-value
Female gender, %	18%	16%	19%	NS
Age, years	59 ± 12	60 ± 12	59 ± 12	NS
Risk Factors				
Hypertension, %	56%	52%	58%	NS
Diabetes, %	17%	17%	17%	NS
Dyslipidemia, %	86%	87%	86%	NS
Obesity, %	21%	16%	24%	0.07
Smoking, %	7%	2%	9%	<0.01
Sedentary, %	74%	67%	78%	0.03
Health care insurance system				
Public health system, %	21%	25%	19%	NS
Private health system, %	78%	74%	80%	NS
No health insurance, %	1%	1%	1%	NS
Intervention				
Cardiac surgery, %	35%	38%	33%	NS
Angioplasty, %	49%	50%	48%	NS
Medical, %	16%	13%	19%	NS
Educational Level				
Primary or Secondary, %	15%	11%	16%	NS
Technical, %	17%	14%	17%	NS
University, %	69%	75%	67%	NS
Quality of life survey (SF-36)				
SF-36 on physical health score	74 ± 17	81 ± 12	70 ± 18	<0.0001
SF-36 on emotional health score	68 ± 22	76 ± 18	64 ± 21	<0.0001

Data are mean ± SD, except where indicated. T-tests were used to determine differences between the non-adherent and adherent group. *Adherence was defined as attendance at all 36 sessions of the phase II cardiac rehabilitation program. NS = not significant; SF-36 = Short Form 36 health survey.

program was 33%. No significant differences were observed between men and women (35% versus 29%, respectively). Overall, RF prevalence was: dyslipidemia 86%, hypertension 56%, diabetes 17%, obesity 21%, smoking 7%, insulin resistance 13%, 74% sedentary lifestyle, and 25% family history of CHD. Thirty-five percent of patients had coronary artery bypass surgery, 49% percutaneous coronary revascularization, either balloon angioplasty or stenting, and 16% only received optimal medical treatment.

A significant difference was observed between the following conditions and adherence (*i.e.*, "worse" adherence"): smoking ($p < 0.01$), sedentary behavior prior to the Phase-II-CR program ($p = 0.03$), and SF-36 score < 50 in physical health ($p < 0.0001$).

As shown in **Figure 1**, a better rate of adherence was observed in patients older than 50 years old (37% adherence rate compared with 23% in patients 17 to 50 years old; $p = 0.05$). When the group was analyzed by smoking status, better adherence was demonstrated in non-smokers versus smokers: 35% versus 10% ($p < 0.01$).

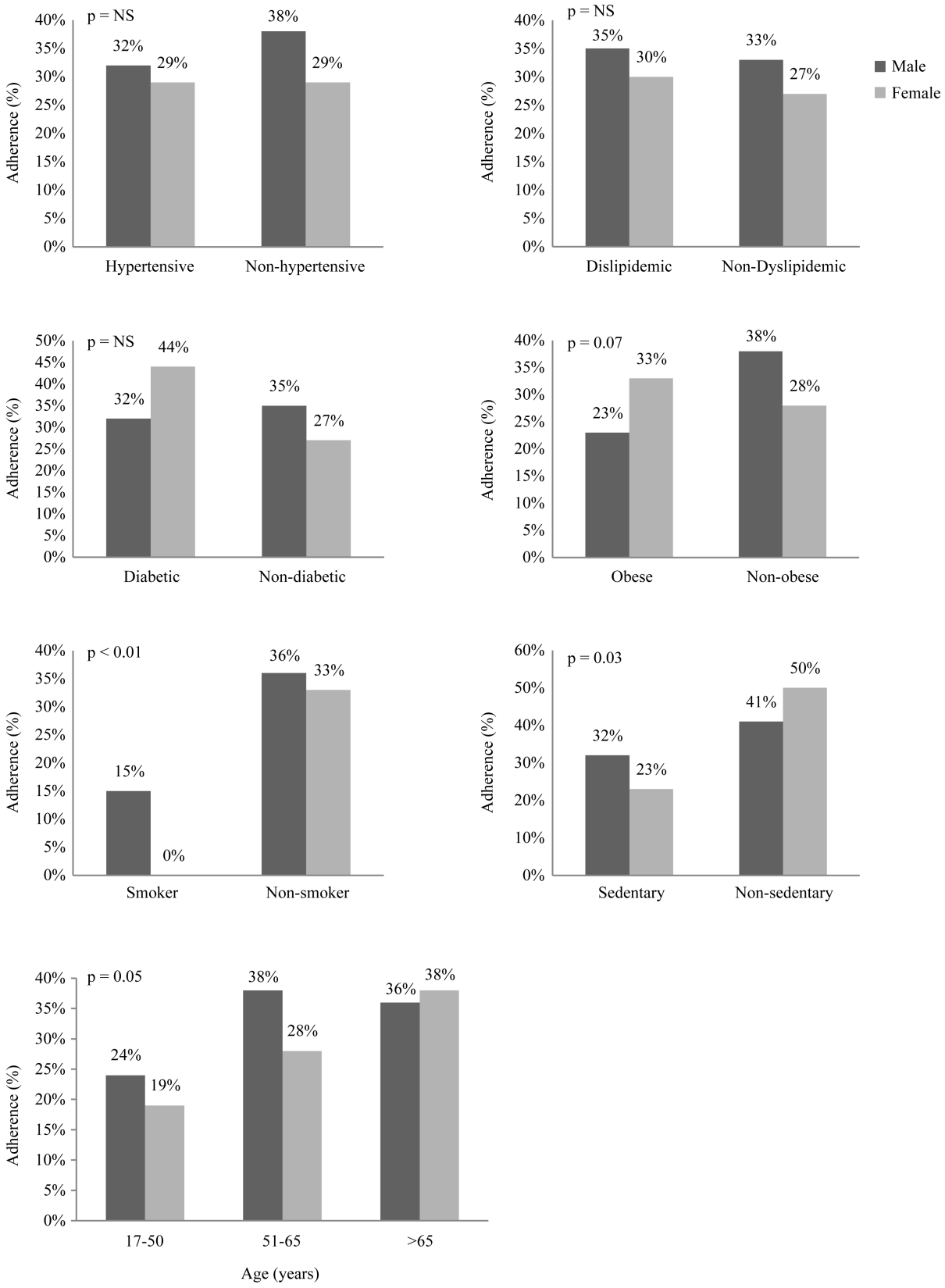

Figure 1. Rates of adherence by gender and cardiovascular risk factors.

Likewise, a higher adherence was observed in the previously active versus previously sedentary subjects (42% vs 30%; $p = 0.03$). The adherence rate was lower in previous sedentary women (23%) than men (32%). Moreover, a higher adherence rate was seen in non-obese compared to obese patients (36% vs 25%, respectively), however it did not reach statistical significance ($p = 0.07$).

When analyzing the adherence according to the clustering of RF, no significant differences were observed, although a trend toward worse adherence was seen in subjects presenting with more than 4 risk factors (35% in subjects with 2 to 4 RFs versus 19% in those with >4 RFs; $p = 0.06$) (**Figure 2**).

The logistic regression model adjusted for age, gender, RFs, and health insurance system showed the following conditions as predictors of non-adherence: smoking (odds ratio [OR] = 4.39, $p = 0.02$), and previous physical sedentary behavior (OR = 1.58; $p = 0.05$) (**Table 2**). When SF-36 score was included in the model, SF36 < 50 score on physical health and smoking remained as the only predictors of non-adherence (OR = 11.47, $p < 0.0001$ and OR = 4.41, $p = 0.02$, respectively) (**Table 3**).

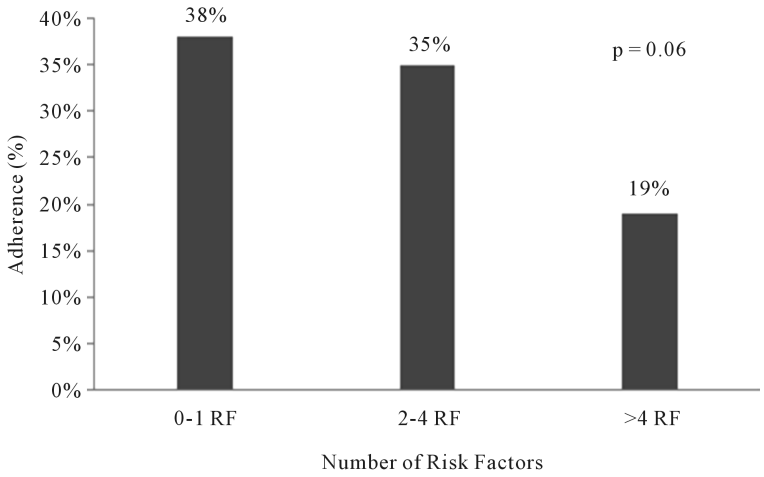

Figure 2. Adherence according to clustering of cardiovascular risk factors.

Table 2. Adjusted logistic regression model showing factors associated with adherence (adjusted for age, gender, risk factors, and health insurance).

	Odds Ratio (95% CI)	*p*-value
Male gender	1.00	Reference
Female gender	1.17 (0.67 - 2.04)	NS
Age (continuous)	1.0 (0.98 - 1.01)	NS
Smoking (−)	1.00	Reference
Smoking (+)	4.39 (1.29 - 14.92)	0.02
Dyslipidemia (−)	1.00	Reference
Dyslipidemia (+)	0.96 (0.51 - 1.78)	NS
Obesity (−)	1.00	Reference
Obesity (+)	1.64 (0.95 - 2.82)	0.07
Previously Active	1.00	Reference
Previously Sedentary	1.58 (1.00 - 2.51)	0.05

Odds ratio >1 indicates predictors of non-adherence; odds ratio <1.00 indicates predictors of adherence. CI = confidence interval; NS = not significant.

Table 3. Fully adjusted logistic regression model showing factors associated with adherence (adjusted for age, gender, risk factors, health insurance and SF-36 score).

	Odds Ratio (95% CI)	*p*-value
Male gender	1.00	Reference
Female gender	0.44 (0.21 - 0.92)	0.03
Age (continuous)	0.99 (0.97 - 1.01)	NS
Smoking (−)	1.00	Reference
Smoking (+)	4.41 (1.25 - 15.62)	0.02
Dyslipidemia (−)	1.00	Reference
Dyslipidemia (+)	0.81 (0.4 - 1.63)	NS
Obesity (−)	1.00	Reference
Obesity (+)	1.58 (0.88 - 2.83)	NS
Previously Active	1.00	Reference
Previously Sedentary	1.04 (0.62 - 1.74)	NS
SF 36 score >50 both	1.00	Reference
Physical Health score <50	11.47 (3.99 - 32.99)	<0.0001
Mental Health score <50	1.61 (0.82 - 3.19)	NS

Odds ratio >1 indicates predictors of non-adherence; odds ratio <1.00 indicates predictors of adherence. CI = confidence interval; NS = not significant.

4. Discussion

In our study adherence rates to a Phase-II-CR program were low. Patient RFs, such as score in the SF-36 < 50, active smoking, and previous physical inactivity favored non-adherence to the program rather than health system-related issues. On the other hand, there were no risk factors that influenced a better adherence in our population. This study emphasizes the need for prospective studies to determine all the possible factors—including patient, referring physician, health center, and health system-related factors, that may influence adherence and the success of this type of intervention.

Current guidelines assign Phase-II-CR an indication IA for use in secondary prevention [8] [20]. Phase-II-CR has been defined in the literature as the "clinical application of preventive care through a multidisciplinary professional approach to a comprehensive risk reduction and global long-term care of cardiovascular patients" [21]. Such programs include not only physical activity but also education about secondary prevention. This comprehensive concept underscores the need for the patient to adhere to the program. Since improvements in aerobic capacity, RF control, and healthy lifestyles habits are not achieved immediately, the current recommendation is that Phase-II-CR (outpatient) should last at least 36 sessions (a minimum of 3 times/week for approximately 3 months).

Our study is the first to report the adherence to a Phase-II-CR program in our country and Latin America. Overall adherence to our program was 33%, which is significantly lower than the 51% reported in a recent study of 4412 participants in a group of CR centers in Wisconsin [22]. In that study, adherence was significantly better in men than women, unlike our study where there was no difference by sex. In another, smaller study from Australia (n = 284), Worcester and colleagues reported adherence rates of 76% in men and 79% in women [23]. The poor adherence rates observed in our patients stresses the importance of recognizing the contributing factors that are specific to our Latin population. We could speculate that the poor adherence observed in our program may have been due to the overall poor knowledge of the Chilean population about the benefits of this type of treat-

ment. In this regard, it is important to emphasize that Phase-II-CR is not typically included in secondary prevention programs in Chile: not only are patients generally unaware of its existence and benefits but also it is not a compelling indication for physicians managing these patients. Therefore, recognizing and understanding the factors influencing adherence to these programs when these programs become more widely available in countries such as ours, in both public and private health system facilities, are essential.

Among the most important factors for non-adherence to the program, the low score in SF-36 (<50 points) in physical and mental health according to international studies [19]) stood out. Our results highlight the importance of the assessment of quality of life in CR programs. Other factors that negatively influenced adherence in our group were obesity, physical inactivity and smoking. In obese subjects adherence was 25%, with an OR of 1.64 as a predictor of "lower adherence". This information is extremely important given the high prevalence of overweight and obesity worldwide [17]. Recent evidence has shown that individual or group nutritional counseling within a CR program favorably affects adherence and physical activity compliance [22]. In our center, patients have free access to a nutritionist within the preventive cardiology unit. However, we did not include this variable in the analysis as all our patients are seen by the nutritionist. In view of the findings, it is an important variable that may have a positive impact on adherence and should be included in future research. With respect to obesity, it was also interesting to realize that, when adjusting for SF-36 health score in the regression model, the significance of obesity as a negative prognostic factor for adherence was lost. This finding suggests that these two variables may discriminate the same kind of subject, *i.e*. patients who are more resistant to lifestyle changes. Of note, morbidly obese patients in our group had a lower score on SF-36 health than non-obese patients (data not shown).

The harmful effect of smoking on adherence in both men and women, has been previously reported in the literature [14] [23] [24]. In our sample, only 7% of the subjects were smokers. This low prevalence was anticipated as all subjects were secondary prevention patients in whom rates of smoking (especially in the first year after the cardiovascular event) are low. Phase-II-CR programs put special emphasis on smoking cessation. In general, it is important to ensure that CR programs handle smoking cessation carefully and sensitively so as not to alienate smokers who may feel marginalized or defensive about their habit. It has been reported that smokers are more likely to remain in CR programs when the difficulties of quitting are known and handled with care [25]. Ideally, both obesity and smoking should be managed with complementary therapies and multidisciplinary teams. Non-adherence rates could improve when CR programs provide comprehensive care including smoking cessation and obesity management.

Studies have shown that historical sedentary lifestyle also influences non-adherence. In women, dropout from CR programs has been strongly associated with previously complete physical inactivity [23]. These data are similar to our results in which sedentary women were less adherent to the program. Physical inactivity prior to admission to the CR program coupled with obesity and smoking, suggest that dropout from Phase-II-CR programs may be related to a reluctance to make lifestyle changes.

Our results also demonstrated that age >65 years old was not a factor associated with less adherence. In contrast, when subjects were stratified into age tertiles, we observed that subjects >50 years were more adherent than participants aged 17 to 50 years. These data differ from other studies [23] [26] showing that older adults are less adherent, likely due to factors such as difficulties in transportation, dependence on third parties to comply with the program, and other comorbidities affecting mobility [27]. Our positive findings in older patients may have been related to specific characteristics of our program, such as proximity of the center to public transportation, reminder calls to promote attendance, and individual care of the physical therapist to address joint-related pains.

Participants in our study had a high prevalence of hypertension and dyslipidemia in comparison to other studies [23] [28]. However, they did not influence adherence as it has been previously shown in one study [23]. Dyslipidemia, particularly, hypercholesterolemia and hypertension are conditions that are easily treated with diet and medication. In our program at admission and at the end of 36 sessions, all participants complete a survey that tests them on their knowledge about cardiovascular RF knowledge. In this survey, we make it clear that dyslipidemia is one of the most important RFs for atherosclerosis. All participants had well-controlled blood pressure levels and approximately 80% of them reached LDL-C <70 mg/dL. Therefore, it might be speculated that dyslipidemia and blood pressure are not barriers to compliance within our program. These results contrast with what happened in smokers and obese patients in our group—two factors that are highly dependent on patients' motivation to achieve lifestyle changes without help of medications. We did not measure adherence to lipid-

lowering medications; however, the high proportion of patients achieving LDL-C <70 mg/dL suggests many patients were taking statins. Unlike other studies [23] [28], diabetes was not a predictive factor of non-adherence. This may have been due to the low prevalence of diabetes in our population.

Finally, we were interested on the effect of RF clustering on adherence. Patients with >4 RFs were less adherent to the Phase-II-CR program than those with 0 to 4 RF. With these results we might speculate that patients with >4 RFs could have felt that lifestyle changes were impossible to achieve and/or they could have been less aware of their illness.

Our study has several limitations. The main caveat is that this is a retrospective study and it was done in a single center and therefore, biased to sample selection. The sample size may have influenced some factors, such as the low prevalence of diabetes. Moreover, in the statistical analysis only the factors which were anticipated by the literature as predictors of adherence were included in the database.

5. Conclusion

Future studies with larger number of subjects or multicenter studies could identify other predisposing factors to non-adherence. Until then, it is important to raise awareness about the benefits of Phase-II-CR programs, patient expectations, and possible barriers to successful implementation and participation in structured CR programs in order to improve adherence rates.

Disclosures

The authors declare that there is no conflict of interest regarding the publication of this paper. All authors have read and approved the manuscript.

References

[1] INE (2010) Estadísticas Vitales: Informe Anual 2008. http://www.ine.cl/canales/menu/publicaciones/calendario_de_publicaciones/pdf/21_12_10/vit_08211210.pdf

[2] Nazzal, C. and Alonso F.T. (2011) Incidencia y letalidad por infarto agudo del miocardio en Chile: 2001-2007. *Revista médica de Chile*, **139**, 1253-1260. http://dx.doi.org/10.4067/S0034-98872011001000002

[3] Lawler, P.R., Filion, K.B. and Eisenberg, M.J. (2011) Efficacy of Exercise-Based Cardiac Rehabilitation Post-Myocardial Infarction: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *American Heart Journal*, **162**, 571.e2-584.e2. http://dx.doi.org/10.1016/j.ahj.2011.07.017

[4] Smith S.C., Benjamin E.J., Bonow R.O., Braun L.T., Creager M.A., Franklin B.A., *et al.* (2011) AHA/ACCF Secondary Prevention and Risk Reduction Therapy for Patients with Coronary and Other Atherosclerotic Vascular Disease: 2011 Update: A Guideline from the American Heart Association and American College of Cardiology Foundation endorsed by the World Heart Federation and the Preventive Cardiovascular Nurses Association. *Circulation*, **124**, 2458-2473. http://dx.doi.org/10.1161/CIR.0b013e318235eb4d

[5] Hsu, C.J., Chen, S.Y., Su, S., Yang, M.C., Lan, C., Chou, N.K., *et al.* (2011) The Effect of Early Cardiac Rehabilitation on Health-Related Quality of Life among Heart Transplant Recipients and Patients with Coronary Artery Bypass Graft Surgery. *Transplantation Proceedings*, **43**, 2714-2717. http://dx.doi.org/10.1016/j.transproceed.2011.04.025

[6] Acevedo, M., Krämer, V., Bustamante, M.J., Yañez, F., Guidi, D., Corbalán, R., *et al.* (2013) Rehabilitación cardiovascular y ejercicio en prevención secundaria. *Revista médica de Chile*, **141**, 1305-1312. http://dx.doi.org/10.4067/s0034-98872013001000010

[7] Hammill, B.G., Curtis, L.H., Schulman, K.A. and Whellan, D.J. (2010) Relationship between Cardiac Rehabilitation and Long-Term Risks of Death and Myocardial Infarction among Elderly Medicare Beneficiaries. *Circulation*, **121**, 63-70. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.876383

[8] Suaya, J.A., Shepard, D.S., Normand, S.L., Ades, P.A., Prottas, J. and Stason, W.B. (2007) Use of Cardiac Rehabilitation by Medicare Beneficiaries after Myocardial Infarction or Coronary Bypass Surgery. *Circulation*, **116**, 1653-1662. http://dx.doi.org/10.1161/CIRCULATIONAHA.107.701466

[9] Leon, A.S., Chair, M.S. Franklin, B.A., Costa, F., Balady, G.J., Berra, K.A., *et al.* (2005) An American Heart Association scientific statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention); Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity) in collaboration with the American Association of Cardiovascular and Pulmonary Rehabilitation. Cardiac Rehabilitation and Secondary Prevention of Coronary Heart Disease. *Circulation*, **111**, 369-376. http://dx.doi.org/10.1161/01.CIR.0000151788.08740.5C

[10] Yohannes, A.M., Yalfani, A., Doherty, P. and Bundy, C. (2007) Predictors of Drop-Out from an Outpatient Cardiac Rehabilitation Programme. *Clinical Rehabilitation*, **21**, 222-229. http://dx.doi.org/10.1177/0269215506070771

[11] Santibáñez, C., Pérez-Terzic, C., López-Jiménez, F., Cortés-Bergoderi, M., Araya M.V. and Burdiat, G. (2012) Situación actual de la rehabilitación cardiaca en Chile. *Revista médica de Chile*, **140**, 561-568. http://dx.doi.org/10.4067/S0034-98872012000500002

[12] Scott, L.A., Ben-Or, K. and Allen J.K. (2002) Why Are Women Missing from Outpatient Cardiac Rehabilitation Programs? A Review of Multilevel Factors Affecting Referral, Enrollment, and Completion. *Journal of Women's Health*, **11**, 773-791. http://dx.doi.org/10.1089/15409990260430927

[13] Todaro, J.F., Shen, B.J., Niaura, R., Tilkemeier, P.L. and Roberts, B.H. (2005) Do Men and Women Achieve Similar Benefits from Cardiac Rehabilitation? *Journal of Cardiopulmonary Rehabilitation*, **24**, 45-51.

[14] Jackson, L., Leclerc, J., Erskine, Y. and Linden, W. (2005) Getting the Most Out of Cardiac Rehabilitation: A Review of Referral and Adherence Predictors. *Heart*, **91**, 10-14. http://dx.doi.org/10.1136/hrt.2004.045559

[15] Grundy, S.M., Brewer Jr., H.B., Cleeman, J.I., Smith Jr., S.C. and Lenfant, C. (2004) Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Circulation*, **109**, 433-438. http://dx.doi.org/10.1161/01.CIR.0000111245.75752.C6

[16] Alberti, K.G.M.M., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., *et al.* (2009) Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, **120**, 1640-1645. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.192644

[17] Ministerio de Salud, Pontificia Universidad Católica y Universidad Alberto Hurtado (2011) Encuesta Nacional de Salud. Chile 2009-2010. http://www.redsalud.gov.cl/portal/url/item/99c12b89738d80d5e04001011e0113f8.pdf

[18] Krämer, V., Acevedo, M., Orellana, L., Chamorro, G., Corbalán, R., Bustamante, M.J., *et al.* (2009) Actividad física y potencia aeróbica: Cómo influyen sobre los factores de riesgo cardiovascular clásicos y emergentes? *Revista Médica de Chile*, **137**, 737-745. http://dx.doi.org/10.4067/S0034-98872009000600002

[19] (1998) U.S. Population Norms. United States of America. http://www.sf-36.org/research/sf98norms.pdf

[20] Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., *et al.* (2012) European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (Version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by Representatives of Nine Societies and by Invited Experts). *European Heart Journal*, **33**, 1635-1701. http://dx.doi.org/10.1093/eurheartj/ehs092

[21] Corrá, U., Piepoli, M.F., Carré, F., Heuschmann, P., Hoffmann, U., Verschuren, M., *et al*. (2010) Secondary Prevention through Cardiac Rehabilitation: Physical Activity Counseling and Exercise Training: Key Components of the Position Paper from the Cardiac Rehabilitation Section of the European Association of Cardiovascular Prevention and Rehabilitation. *European Heart Journal*, **31**, 1967-1974. http://dx.doi.org/10.1093/eurheartj/ehq236

[22] Turk-Adawi, K.I., Oldridge, N.B., Tarima, S.S., Stason, W.B. and Shepard, D.S. (2013) Cardiac Rehabilitation Patient and Organizational Factors: What Keeps Patients in Programs? *Journal of the American Heart Association*, **2**, e000418. http://dx.doi.org/10.1161/JAHA.113.000418

[23] Worcester, M.U., Murphy, B.M., Mee, V.K., Roberts, S.B. and Goble, A.J. (2004) Cardiac Rehabilitation Programmes: Predictors of Non-Attendance and Drop-Out. *European Journal of Cardiovascular Prevention and Rehabilitation*, **11**, 328-335. http://dx.doi.org/10.1097/01.hjr.0000137083.20844.54

[24] Oldridge, N.B. and Streiner, D.L. (1990) The Health Belief Model: Predicting Compliance and Dropout in Cardiac Rehabilitation. *Medicine & Science in Sports & Exercise*, **22**, 678-683. http://dx.doi.org/10.1249/00005768-199010000-00020

[25] Wakefield, M., Roberts, L. and Rosenfeld, E. (1997) Smoking Cessation among People with Diabetes: Beliefs and Barriers. *Health Promotion Journal of Australia*, **7**, 46-51.

[26] McGee, H.M. and Horgan, J.H. (1992) Cardiac Rehabilitation Programmes: Are Women Less Likely to Attend? *British Medical Journal*, **305**, 283-284. http://dx.doi.org/10.1136/bmj.305.6848.283-a

[27] Jones, M., Jolly, K., Raftery, J., Lip, G.Y., Greenfield, S. and BRUM Steering Committee (2007) "DNA" May Not Mean "Did Not Participate": A Qualitative Study of Reasons for Non-Adherence at Home- and Centre-Based Cardiac Rehabilitation. *Family Practice*, **24**, 343-357. http://dx.doi.org/10.1093/fampra/cmm021

[28] Dunlay, S.M., Witt, B.J., Allison, T.G., Hayes, S.N., Weston, S.A., Koepsell, E., *et al.* (2009) Barriers to Participation in Cardiac Rehabilitation. *American Heart Journal*, **158**, 852-859. http://dx.doi.org/10.1016/j.ahj.2009.08.010

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Patient Enablement in Chronic Diseases in Primary Health Care, Riyadh City, KSA

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Abstract

Background: Chronic diseases continue to cause high morbidity and mortality in Saudi Arabia. Patients severing from diabetes mellitus, hypertension and associated complications have recently increased and most of these patients find it extremely difficult to understand or cope with their illness. The objective of this study is to determine the level of patients' enablement in chronic disease and its predictors. Methods: A community based cross-sectional study was conducted between December 2014 and January 2015. Six hundred and four (604) Patients attending the Chronic Disease Clinic in Alwazarat Health center were randomly selected to participate in the study. Patients aged 18 years and above, who willingly agreed to participate, were included in the study. Self-reported questionnaire was used to determine patient level of enablement. Descriptive statistics such as mean and median were calculated and binary logistic regression was employed to determine the predictors of patient's enablement to chronic disease. Results: Our results show that five hundred and sixty five (565) out of (604) patients participated in the study with 86.6% response rate. Type 2 diabetes mellitus affecting 40.65% while hypertension affecting 37.79% of the patients in Al wazarat health center. Patient's enablement to chronic disease was very low and ranged between 2.41 and 1.53 out of 5.0. Binary logistic regression shows that age (male: OR; 0.84, 95% CI, 0.72 - 1.04, female. OR; 1.04, 95% CI 0.88 - 1.39), marital status (male: OR; 0.72, 95% CI 0.54 - 1.11, female: OR 1.01; 95% CI 0.82 - 1.29), patient educational level and number of problems discussed with physician and consultation length between male patients and their physician were statistically significant and correlated with patients enablement to chronic disease ($P < 0.05$). Conclusion: This study shows that patient's enablement in chronic disease is very low but constitutes an important arm in patients care management. It should be considered as a measurable patient outcome from healthcare services. More prospective studies on this important topic are highly recommended.

Keywords

Chronic Disease, Cross-Sectional, Logistic Regression, Enablement, Patient Assessment

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1. Introduction

The Global Burden of Disease and Injury study estimated that worldwide death due to chronic conditions is increasing and projected to rise up to about 70% by 2020 [1]-[3]. In Saudi Arabia the improvement in the quality of healthcare provided has shifted and reduces death due to infection diseases with similar increase in the prevalence of death due to chronic conditions such as diabetic mellitus, hypertension and associated complications [4] [5]. Chronic diseases consume a lot of time and efforts from health professions and it is for the benefit of both parties that patients enablement is considered highly [6]. Unlink in acute illness, patient with chronic disease modify or adapt different behaviors than that prior to their chronic illness [7]. Their behavior depends largely on how they accept their illness and how the health care givers organize their services [4]. Although a lot of guidelines were published on the management of different chronic disease such as diabetes, hypertension and asthma but there is a gap in the effective patient involvement of their own problems [8].

It has been shown that in many studies patients enablement plays very significant part in treatment of chronic conditions [9] [10]. Patient enablement is defined as the extent to which a patient is capable of understanding and coping with his or her health issues [11]. The concept of enablement was further focused in the general practice setting, to the extent of the patient's own feelings of enablement about his/her condition after the medical consultation [12]. In general practice, the doctor patient consultation is a situation where different issues concerning patient health are encountered [13]. In many occasions the patients' understanding, capability of coping with their illness and taking care of themselves are all left un-defined in the hand of the medical service provider [14]. Patients present to the primary care settings for various reasons, some of them come for acute short trivial problems, and others may come frequently for long standing chronic diseases [2] [15]. Health promotion definition was changing over the years to embrace the process of enabling people to increase control over their health and its determinants, and thereby improve their health (Michael P. O'Donnell, 2009) [16]. It is expected that health promotion is one of the important part of all these consultation with special considerations to chronic diseases [17]. The more enabled the patients about understanding their illness, the better the outcome for both patients and the healthcare providers.

This study's objective is to assess the level of patients' enablement in primary care chronic disease clinics and determine its main predictors.

2. Methods

2.1. Study Design

An observational cross-sectional study was carried out in Al wazarat Health Center in Riyadh between December 2014 and January 2015.

2.2. Study Area and Population

Al Wazarat health center is located in the Middle of Riyadh city. It runs many clinics such chronic disease clinic which provides primary care services to Riyadh military personnel, civil employed personnel in the military organizations and their dependents. Adult patients aged above 18 years with history of chronic illness such as diabetes, hypertension and bronchial asthma who willingly agree to participate in the study were included. Only patients who have been seen in the center for more than 6month were allowed to participate in the study. Patients who did not consented to participate in the study and those with who were severely ill were excluded from the study.

2.3. Sample Size and Sampling Method

A sample size of 669 was calculated using Epi Info 2000 (ATLANTIS 2004) [18]. A convenient sampling method was adapted because chronic disease patients may lack interest to participate in studies due to their chronic conditions.

2.4. Study Variables

The study variables were divided into dependent or outcome variable such as patient chronic disease enablement while the independent variables were the socio-demographic characteristics such age, gender, marital status and

income, employment status and educational level.

2.5. Study Instrument (Questionnaire)

The questionnaire was developed by authors form literature and experts from the department of family and community medicine department. The questions used are close ended in the Yes and No format. It consisted of part one which contain the socio-demographic characteristics and part two had questions about patient's enablement and chronic diseases such as diabetes Miletus, hypertension, hypothyroidism and asthma. It was pre-tested on 20 participants with different educational level, validated and then translated into Arabic language by two translators.

2.6. Data Collection

Data was collected using self-reported questionnaire by a train research assistant. It was administered to participants who satisfied the inclusion criteria stated above. All privacy and confidentiality were maintained.

2.7. Data Entry and Analysis

Collected data was entered into SPSS version 18 for windows by research assistant [19]. Six hundred and four patients were approached for the study (604), and five hundred and sixty five (565) participated in the study. Forty one study instruments were found to have incomplete data and were removed, the remaining 524 instrument were analyzed. Initial descriptive analysis included frequency, mean and median and percentages were carried out. Binary logistic regression was used to predict the association between variables. The dependent variable was enablement score and socio-demographic characteristics were independent variables.

Ethical approval was obtained before the start of the study. Patient's confidentiality and privacy were maintained throughout the study period.

3. Results

Of the 604 study participants 524 returned completed study questionnaire given a response rate of 86.6%. Majority of the participants were male (58.8%) and the remaining 41.2% were female. Participants with age group greater than 42 years of age were 45.9% as compared to 0.6% for participants aged between 18 and 25 years. Most of the participants had one form of education or other (89.7%) while only 10.3% were illiterate. Almost two third of the participants 65.1% were employed while the remaining 34.9% were unemployed. **Table 1** shows the details of socio-demographic characteristics as described above. Majority of patients had Diabetes Milieus (213, 40.65%), followed by hypertension (198, 37.79%) while hypothyroidism was the disease with the least prevalence rate of (16, 3.05%) as shown in (**Table 2**). The highest incidence rate was 0.41 for DM compared with 0.03 for hypothyroidism.

Table 3 shows the results of patient's median enablement scores for some socio-demographic characteristics and consultations parameters. Median enablement score for male participants was 2.39 as compared to 2.03 for females. Out of the total 524 participants 203 or 38.7% knew their doctors very well and had a median enablement score of 2.41. One hundred and eleven or 21.2% of the participants knew their doctors well with median enablement score of 2.30. The lest median enablement score was 1.63 for the participants who did not know their doctors at all. Participants who discussed one problem with their doctors had highest median enablement score of 2.38 as compared to lowest median enablement score of 1.53 for the participants who discussed more than three problems with their doctors. Patients with consultations time of 12 to 17 minutes had higher median enablement score of 2.31 as compared to a lower median score of 1.82 for those who spent less than five minutes with their doctors.

Binary logistic regressions model was utilized t to predict the association between patient enablement to chronic disease (Dependent variable) and socio-demographic characteristics and patients consultations character as (independent variables). Patient educational level number of problems (related to their chronic disease) discussed with their physician and consultation length was statistically significant (**Table 4**) while patients age, marital status, employment status and patients knowledge of their physician were not statistically significant.

Table 4 describes the results of patients enablement in chronic disease as associated with their corresponding show demographic characteristics. Patients' age and marital status were found to be statistically insignificant

with p value of 0.315 and 0.084 respectively. Patient educational level for male and female participants was found to be statistically significant with p vale of 0.021 and 0.001 respectively. Patient consultation length has been found to be statistically associated with their enablement score level with a p value of 0.048. Number of problems discussed between patients and their doctors during consultation was also found to be statistically significant with their median enablement score with a p value of 0.002 and 0.004 for males and females participants.

Table 1. Socio-Demographic characteristics of participants (n = 524).

Variable	N	%
Gender		
Female	216	41.2
Male	308	58.8
Total	524	100
Age (Years)		
18 - 25	034	06.49
26 - 33	092	17.56
34 - 41	156	29.77
>42	242	45.92
Total	524	100
Marital status		
Married	293	55.92
Single	58	11.07
Divorced	127	24.24
Widow	29	5.54
Widower	17	3.24
Total	524	100
Education level		
No formal schooling	54	10.31
Primary school	148	28.24
Secondary school	169	32.25
University	134	25.57
Postgraduate	19	3.63
Total	524	100
Employment Status		
Not employed	183	34.92
Employed	341	65.08
Total	146	100

Table 2. Types of chronic disease and number of patients in follow up with Chronic Diseases Clinic in Wazarat Family Medicine Center (n = 524).

Chronic Disease	Patient Number (N)	Percentage (%)	Prevalence Rate	95% Confidence Interval
Diabetes Mellitus	213	40.65	0.4065	0.3537 - 0.4649
Hypertension	198	37.79	0.3779	0.3271 - 0.4343
Bronchial Asthma	42	8.02	0.08015	0.05777 - 0.10834
hypothyroidism	16	3.05	0.03053	0.01745 - 0.0495955
Other chronic illness	55	10.50	0.105	0.0791 - 0.1366
Total	524	100		

Table 3. Consultation parameters and calculated patients enablement score (n = 524).

	Patient No	Percentage (%)	Median Enablement Score
Gender			
Female	216	41.2	2.03
Male	308	58.8	2.39
Total	**524**	**100**	
Patients level of knowing the doctor			
Very well	203	38.7	2.41
Well	111	21.2	2.30
Fairly well	86	16.4	1.99
Not at all	124	23.7	1.63
Total	**524**	**100**	
No of problems discussed by participants			
1	128	24.5	2.38
2 - 3	349	66.6	2.1
>3	47	8.9	1.53
Total	**524**	**100**	
Length of consultation (minutes)			
0 - 5	58	11.1	1.82
6 - 11	259	49.4	1.93
12 - 17	122	23.3	2.31
>17	85	16.2	1.73
Total	**524**	**100**	

Table 4. Association between Patient Enablement in chronic disease and Patients socio-demographic and consultation characters.

Predictor	Male (Odds ratio with 95% CI)	p	Female (Odds ratio with 95% CI)	p
Age	0.84 (0.72 - 1.04)	0.435	1.04 (0.88 - 1.39)	0.315
Education	1.25 (1.02 - 1.83)	0.021*	1.18 (0.93 - 1.25)	0.001*
Marital status	0.72 (0.54 - 1.11)	0.712	1.01 (0.82 - 1.29)	0.084
Consultation length	0.99 (0.83 - 1.29)	0.048*	0.92 (0.87 - 1.01)	0.223
Knowing the Physician	1.00 (0.99 - 1.07)	0.062	1.03 (1.00 - 1.28)	0.082
No of Problems discussed with the physician	0.86 (0.74 - 1.05)	0.002*	0.98 (0.75 - 1.02)	0.004*
Employment status	1.55 (1.32 - 2.07)	0.142	1.65 (1.48 - 1.85)	0.245

*p-value less than 0.05 is considered statistically significant.

4. Discussion

Patients enablement which is about patients ability to understand their sickness and cope with it, in clinical practice generally depend on two important elements namely "holism" which, is an approach to construction of diagnoses and patient-centered care which is defined as the use of adequate consulting skills in identifying patients' priorities and concerns and the appropriate involvement of patients in making decisions about their care.

This study found that the average total chronic disease patient's enablement score was very low and ranged between 2.41 and 1.53 out of 5.0, these values can also be compared to similar finding by John GR and colleges who found an enablement score of about 44.1% [20].

Male participants had high enablement compared to their female counterpart in the study. Patients who knew their doctors before consultation were also more enabled compared to those who did not know their doctors before consultation. Patient's enablement was found to be affected by the consultation language, consultation time, number of problems discussed between the patients and their physician and ethnic differences [21]-[23]. In our study Arabic language was the main consultation language and the embalmment score was lower (2.41) when compared with other similar studies in UK, USA and Poland. Patients enablement was higher in similar study in UK (3.1) and (3.6) in Poland [24]. It is important however to put the educational level of patient and their culture into consideration when comparing patients chronic disease enablement in Saudi Arabia with their counterpart in UK and USA. Saudi Arabia is conservative society as compared to other western countries like UK, this significantly affecting patient's doctor relationship and is clearly reflected in low patient's enablement. Patients in UK tend to be more aware of their treatment right during consultation and as such engaged more freely with their health care provider than in our study [25].

The median score for patient enablement was found to be between 2.41 to 1.53. It was clearly affected by patient's doctor relationship, length of consultation and number problems discussed by the patients with his physician. Patients who knew his physician very well had the highest enablement score compared to those who did not knew their physician at all, this result was similar to study conducted by other researcher [26]. Those who had average consultation time of 12 - 17 minutes same to have the highest enablement score (12 - 17, 2.31) compared to those with short consultation time of (0 - 5, 1.82). From **Table 4** above it is also obvious that the number of problems discussed by patients with their physician also significantly affected the patient chronic disease enablement.

Our findings are important and can be utilized to improve the healthcare services provided to patients with chronic diseases in Saudi Arabia and the gulf region as well. Improving patient's enablement level will help them to understand and coup more effectively with their chronic disease.

The study findings just like other studies have some limitations which may have affected our results. We have improved the language, contents and design of our study instruments in order to overcome the recall bias. The generalizability of this study may be limited to Al Wazarat health center and other primary care health center as the authors could not conduct a multi-center study due to limitation of resources. The clinical and practical value

of this study is very important to doctors as findings could be utilized for managing future patients with similar chronic disease in Al Wazarat center and other primary health care settings. From the findings patients' consultation time, number of problems to be discussed during consultations and patients doctors' relationship should be highly considered. Patient's clinical outcome depends mainly on their full enablement in discussing their health problems freely with their doctors within a sufficient consultation time and concentration on only one or two health important problems affecting their health.

In conclusion this is one of the first studies to discuss in details patients enablement to chronic disease in the Kingdom of Saudi Arabia. Our findings are important and can be utilized to improve the healthcare services provided to patients with chronic diseases in Saudi Arabia and the gulf region as well. Improving patient's enablement level will help them to understand and cope more effectively with their chronic disease.

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Author's Contribution

Rabaa K Al Momen is the principle investigator, wrote the article and provided essential logistic for the study while Mishaal A and Osama A wrote the study proposal, contributed equally in data collection, analysis and interpretation. All authors read, edited and agreed to the manuscript before submission for publication.

Conflict of Interest

Authors have no conflict of interest to declare.

References

[1] Murray, C.J. and Lopez, A.D. (1997) Alternative Projections of Mortality and Disability by Cause 1990-2020: Global Burden of Disease Study. *The Lancet*, **349**, 1498-1504. http://dx.doi.org/10.1016/S0140-6736(96)07492-2

[2] Mathers, C.D. and Loncar, D. (2006) Projections of Global Mortality and Burden of Disease from 2002 to 2030. *PLoS Medicine*, **3**, e442. http://dx.doi.org/10.1371/journal.pmed.0030442

[3] Gensichen, J., Serras, A., Paulitsch, M., Rosemann, T., König, J., Gerlach, F. and Petersen, J. (2011) The Patient Assessment of Chronic Illness Care Questionnaire: Evaluation in Patients with Mental Disorders in Primary Care. *Community Mental Health Journal*, **47**, 447-453. http://dx.doi.org/10.1007/s10597-010-9340-2

[4] Hanan, A.-A. and Roland, M. (2005) Quality of Primary Health Care in Saudi Arabia: A Comprehensive Review. *International Journal for Quality in Health Care*, **17**, 331-346. http://dx.doi.org/10.1093/intqhc/mzi046

[5] Whiting, D.R., Guariguata, L., Weil, C. and Shaw, J. (2011) IDF Diabetes Atlas: Global Estimates of the Prevalence of Diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, **94**, 311-321.

[6] Wensing, M., van Lieshout, J., Jung, H.P., Hermsen, J. and Rosemann, T. (2008) The Patients Assessment Chronic Illness Care (PACIC) Questionnaire in The Netherlands: A Validation Study in Rural General Practice. *BMC Health Services Research*, **8**, 182.

[7] Novalis, P.N., Rojcewicz, S.J. and Peele, R. (1993) Clinical Manual of Supportive Psychotherapy. American Psychiatric Publishing.

[8] Brusse, C.J. and Yen, L.E. (2013) Preferences, Predictions and Patient Enablement: A Preliminary Study. *BMC Family Practice*, **14**, 116. http://dx.doi.org/10.1186/1471-2296-14-116

[9] Rogers, A., Kennedy, A., Nelson, E. and Robinson, A. (2005) Uncovering the Limits of Patient-Centeredness: Implementing a Self-Management Trial for Chronic Illness. *Qualitative Health Research*, **15**, 224-239. http://dx.doi.org/10.1177/1049732304272048

[10] Shaw, A., Ibrahim, S., Reid, F., Ussher, M. and Rowlands, G. (2009) Patients' Perspectives of the Doctor-Patient Relationship and Information Giving across a Range of Literacy Levels. *Patient Education and Counseling*, **75**, 114-120. http://dx.doi.org/10.1016/j.pec.2008.09.026

[11] Andén, A., Andersson, S.-O. and Rudebeck, C.-E. (2009) To Make a Difference—How GPs Conceive Consultation Outcomes. A Phenomenographic Study. *BMC Family Practice*, **10**, 4. http://dx.doi.org/10.1186/1471-2296-10-4

[12] Rööst, M., Zielinski, A., Petersson, C. and Strandberg, E.L. (2015) Reliability and Applicability of the Patient Enablement Instrument (PEI) in a Swedish General Practice Setting. *BMC Family Practice*, **16**, 31.

http://dx.doi.org/10.1186/s12875-015-0242-9

[13] Kaplan, S.H., Greenfield, S. and Ware Jr., J.E. (1989) Assessing the Effects of Physician-Patient Interactions on the Outcomes of Chronic Disease. *Medical Care*, **27**, S110-S127. http://dx.doi.org/10.1097/00005650-198903001-00010

[14] Kurosawa, S., Matsushima, M., Fujinuma, Y., Hayashi, D., Noro, I., Kanaya, T., Watanabe, T., Tominaga, T., Nagata, T. and Kawasaki, A. (2012) Two Principal Components, Coping and Independence, Comprise Patient Enablement in Japan: Cross Sectional Study in Tohoku Area. *The Tohoku Journal of Experimental Medicine*, **227**, 97-104. http://dx.doi.org/10.1620/tjem.227.97

[15] Glasgow, R.E., Wagner, E.H., Schaefer, J., Mahoney, L.D., Reid, R.J. and Greene, S.M. (2005) Development and Validation of the Patient Assessment of Chronic Illness Care (PACIC). *Medical Care*, **43**, 436-444. http://dx.doi.org/10.1097/01.mlr.0000160375.47920.8c

[16] O'Donnell, M.P. (2009) Definition of Health Promotion 2.0: Embracing Passion, Enhancing Motivation, Recognizing Dynamic Balance, and Creating Opportunities. *American Journal of Health Promotion*, **24**, 4. http://dx.doi.org/10.4278/ajhp.24.1.iv

[17] Sprangers, M.A. and Aaronson, N.K. (1992) The Role of Health Care Providers and Significant Others in Evaluating the Quality of Life of Patients with Chronic Disease: A Review. *Journal of Clinical Epidemiology*, **45**, 743-760. http://dx.doi.org/10.1016/0895-4356(92)90052-o

[18] Atlantis, T. (2004) Association of Outcome with Early Stroke Treatment: Pooled Analysis of ATLANTIS, ECASS, and NINDS rt-PA Stroke Trials. *The Lancet*, **363**, 768-774. http://dx.doi.org/10.1016/S0140-6736(04)15692-4

[19] Carver, R. and Nash, J. (2011) Doing Data Analysis with SPSS: Version 18.0. Cengage Learning, Belmont.

[20] Howie, J., Heaney, D.J., Maxwell, M. and Walker, J.J. (1998) A Comparison of a Patient Enablement Instrument (PEI) against Two Established Satisfaction Scales as an Outcome Measure of Primary Care Consultations. *Family Practice*, **15**, 165-171. http://dx.doi.org/10.1093/fampra/15.2.165

[21] Howie, J.G., Heaney, D.J., Maxwell, M., Walker, J.J., Freeman, G.K. and Rai, H. (1999) Quality at General Practice Consultations: Cross Sectional Survey. *BMJ*, **319**, 738-743. http://dx.doi.org/10.1136/bmj.319.7212.738

[22] McKinstry, B., Walker, J., Blaney, D., Heaney, D. and Begg, D. (2004) Do Patients and Expert Doctors Agree on the Assessment of Consultation Skills? A Comparison of Two Patient Consultation Assessment Scales with the Video Component of the MRCGP. *Family Practice*, **21**, 75-80. http://dx.doi.org/10.1093/fampra/cmh116

[23] Adžić, Z.O., Katić, M., Kern, J., Lazić, Đ., Nekić, V.C. and Soldo, D. (2008) Patient, Physician, and Practice Characteristics Related to Patient Enablement in General Practice in Croatia: Cross-Sectional Survey Study. *Croatian Medical Journal*, **49**, 813-823. http://dx.doi.org/10.3325/cmj.2008.49.813

[24] Pawlikowska, T., Nowak, P., Szumilo-Grzesik, W. and Walker, J. (2002) Primary Care Reform: A Pilot Study to Test the Evaluative Potential of the Patient Enablement Instrument in Poland. *Family Practice*, **19**, 197-201. http://dx.doi.org/10.1093/fampra/19.2.197

[25] Howie, J.G., Heaney, D.J., Maxwell, M., Walker, J.J. and Freeman, G.K. (2000) Developing a "Consultation Quality Index" (CQI) for Use in General Practice. *Family Practice*, **17**, 455-461. http://dx.doi.org/10.1093/fampra/17.6.455

[26] Gray, D.P., Evans, P., Sweeney, K., Lings, P., Seamark, D., Seamark, C., Dixon, M. and Bradley, N. (2003) Towards a Theory of Continuity of Care. *Journal of the Royal Society of Medicine*, **96**, 160-166. http://dx.doi.org/10.1258/jrsm.96.4.160

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Prevalence and Characteristics of Treatment-Resistant Hypertension among Hypertensive Japanese Outpatients

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Abstract

Aims: To retrospectively investigate the prevalence and characteristics of treatment-resistant hypertension (R-HT) among consecutive hypertensive outpatients, since patients with R-HT are candidates for catheter-based renal sympathetic denervation (RD). Methods: Consecutive hypertensive outpatients (n = 999) were recruited in our hospital. R-HT patients who were candidates for RD had clinic systolic blood pressure > 160 mmHg despite taking three or more antihypertensive drugs including at least one diuretics at higher than standard doses. Results: Our survey indicated that only 26 patients (2.6%) were potential candidates for renal denervation. Candidates for RD showed a significantly higher age ($P < 0.005$), brain natriuretic peptide level ($P = 0.0001$), urinary albumin/creatinine excretion ratio in spot urine ($P < 0.005$), pulse wave velocity ($P < 0.01$), left ventricular end-diastolic diameter ($P < 0.005$), and interventricular septal thickness ($P < 0.005$) than the other 973 patients. Candidates for denervation had a significantly lower hemoglobin ($P = 0.0001$), serum albumin ($P < 0.001$), eGFR ($P < 0.0005$), plasma renin activity ($P = 0.0001$), and plasma aldosterone level ($P < 0.005$) than other patients, while their urinary sodium/creatinine ratio was higher, indicating that patients with R-HT appeared to have a high salt intake. Conclusion: Our retrospective clinical survey indicated that only 0.5% of Japanese hypertensive outpatients (5/999 patients) were candidates for RD. Therefore, establishment of hypertension cohort network will be essential to recruit R-HT patients for RD.

Keywords

Treatment-Resistant Hypertension, Catheter-Based Renal Sympathetic Denervation, Human

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1. Introduction

Development of better antihypertensive drugs during the last 40 years has led to marked improvement of outcomes and there is a trend for the mean blood pressure (BP) to decrease in Japan and worldwide. However, the prevalence of hypertension is still increasing in developed countries as the population is aging rapidly. Hypertension remains the single most important lifestyle-related disease, and it is estimated that there are approximately 43 million persons with HT in Japan [1]. Including people with high normal blood pressure, the so-called spare group of HT, the total number could be more than 60 million. Therefore, management of hypertension is essential for preventing cardiovascular disease both in people with hypertension and those with high normal blood pressure, as has been demonstrated by many clinical studies of antihypertensive drugs.

It is sometimes very difficult to manage hypertension, which is known as treatment-resistant hypertension (R-HT), and there may be poor control of both ambulatory and home BP despite using 5 or 6 antihypertensive drugs. Several studies performed in Australia and Europe have demonstrated that catheter-based renal sympathetic denervation (renal denervation) is effective for R-HT [2]-[4], and this treatment will be investigated in Japan in near future. However, the blinded Symplicity HTN-3 trial did not show a significant decrease of BP after renal denervation compared with the sham control [5], and this result has led to controversy [6].

R-HT is often secondary hypertension, and it is important to screen for underlying causes. If it is not secondary hypertension, it is classified as R-HT of unknown etiology, but the prevalence and characteristics of such hypertension have not been determined in Japanese patients. Also, the characteristics of R-HT patients who are indicated for renal denervation have not been clarified. If this were done, it would be easier to identify patients who could benefit from renal denervation and would allow assessment of the need for this procedure in Japan. Therefore, the present study was performed to determine the characteristics of R-TH patients in Japan and the proportion of such patients who are candidates for renal denervation.

2. Methods

2.1. Study Population

Consecutive outpatients with hypertension (n = 999) attending Fukuoka University Chikushi Hospital (Fukuoka, Japan) from April 2006 to March 2013 were recruited for analysis. Office BP was measured by a standard sphygmomanometer in the sitting position after several minutes sedation and routine physical examination was performed. Patients with a total cholesterol level >220 mg/dL (5.69 mmol/L) or triglycerides >150 mg/dL (3.88 mmol/L) or taking lipid-lowering agents were considered to have dyslipidemia. Patients who were being treated for diabetes mellitus or who had symptoms of diabetes and a fasting glucose level >126 mg/dl (7.0 mmol/L) were considered to have diabetes. Otherwise, the results of a 75 g oral glucose tolerance test were used to diagnose diabetes. Coronary heart disease (CHD) was diagnosed by coronary angiography at the time of enrollment. Patients who were smoking at enrollment were defined as smokers.

Exclusion criteria were stroke, myocardial infarction, other serious cardiovascular complications requiring hospitalization within the previous 6 months, dialysis, acute renal failure, pregnancy and other reasons which made the patient inappropriate for the study in the judgment of the attending physician.

The protocol for this cross-sectional observational study to determine the clinical profile of Japanese R-HT patients was approved by the institutional review board of Fukuoka University Chikushi hospital, and written informed consent was given by all patients before data sampling.

2.2. Definition of Treatment-Resistant Hypertension

2.2.1. R-HT

The guidelines of the European Society of Cardiology and the American Herat Association define R-HT as systolic BP (SBP) ≥ 140/90 mmHg despite lifestyle modification and treatment with 3 or more oral antihypertensive drugs, including a diuretic and 2 or more other antihypertensive drugs at standard or higher doses [7] [8]. In this study, we defined R-HT as an SBP ≥ 140 mmHg despite treatment with 3 oral antihypertensive drugs.

2.2.2. R-HT Candidates for Renal Denervation

In the Symplicity HTN-2 study, R-HT patients indicated for renal denervation were defined by a baseline SBP ≥

160 mmHg despite treatment with 3 antihypertensive drugs including a diuretic or SBP ≥ 150 mmHg in patients with type 2 diabetes [4].

In the present study, we defined R-HT patients who were candidates for renal denervation as having a baseline SBP ≥ 160 mmHg despite treatment with 3 or more oral antihypertensive drugs including 1 diuretic.

For standard antihypertensive therapy, it is recommended that diuretics should be used in combination with calcium channel blockers (CCB) or renin-angiotensin system inhibitors (RAS-I) such as angiotensin II receptor blockers (ARB) and angiotensin-converting enzyme inhibitors (ACE-I) (Guidelines for Management of Hypertension 2014). According to the results of the ASCOT study and the LIFE study, RAS-I including ARB and ACE-I, CCB, and thiazide diuretics are the commonly used antihypertensive agents [1] [2]. In the present study, the definition of R-HT also required patients to be taking at least 3 classes of antihypertensive drugs (RAS-I, CCB, and diuretics). Because the combination of ARB and ACE-I is not strongly recommended based on the results of the ONTARGET study, combination therapy with drugs from these 2 classes was counted as 1 antihypertensive agent [3].

2.3. Laboratory Tests

In addition to the routine laboratory tests, we measured brain natriuretic peptide (BNP). The estimated glomerular filtration rate (eGFR) was calculated using the formula for Japanese patients [4]. To exclude secondary hypertension, a blood sample for endocrine tests was collected from the cubital vein after each patient had rested in the supine position for 30 minutes in the hospital. Various endocrine parameters were measured, including the plasma renin activity (PRA), plasma aldosterone concentration (PAC), plasma adrenaline, plasma noradrenalin, plasma dopamine, serum cortisol, serum thyroid stimulating hormone, and serum free thyroxine. As urine parameters, spot urinary albumin (U-alb), spot urinary sodium (U-Na), spot urinary uric acid, and spot urinary creatinine (U-cre) were determined.

2.4. Brachial-Ankle Pulse Wave Velocity

The brachial-ankle pulse wave velocity (baPWV) was measured by using a PWV/ABI apparatus (Omron Colin Co., Ltd.).

2.5. Transthoracic Echocardiography

An experienced sonographer obtained all echocardiography data, which was interpreted by an experienced staff echocardiographer. The examinations included M-mode, 2-dimensional, conventional Doppler, and color Doppler echocardiography, with the parameters assessed being the left ventricular end-diastolic diameter (LVDd), interventricular septal thickness (IVST), and ejection fraction (EF).

2.6. Statistical Analysis

As items and values to compare , we used gender, smoking history, age, body mass index, white blood cells, hemoglobin (Hb), platelet count, albumin, γ-GTP, hemoglobin A1c (HbA1c), eGFR, uric acid, high-density lipoprotein, low-density lipoprotein, triglycerides, non-esterified fatty acids, C-reactive protein, BNP, baPWV, LVDd, IVST, EF, urinary examination and endocrine parameters.

Statistical analysis was performed using the SAS software package (version 6.12, SAS Institute Inc., Cary, NC, USA). Results are reported as the mean ± standard deviation (SD).

Categorical and continuous variables were compared between the groups by chi-square analysis and analysis of variance, respectively, and $P < 0.05$ was considered significant. Univariate analysis and multivariate analysis (logistic regression) were performed to identify independent factors for R-HT.

3. Results

We screened 999 outpatients with hypertension and found 65 patients (6.5%) who had R-HT. Among them, 26 patients (2.6%) fitted the criteria to be candidates for renal denervation (**Table 1**). These 26 patients included 12 men and 14 women with a mean age of 74.5 years, SBP of 170.8 mmHg, diastolic BP (DBP) of 77.9 mmHg, and pulse rate of 70.8/minute. Twenty-five patients were being treated with ARB, 5 with ACE-I, and 4 with

Table 1. Patients with R-HT who were candidates for renal denervation (renal denervation group).

Patient	Gender	Age, Year	DM	Dyslipidemia	CHD history	Smoking	SBP (mmHg)	DBP (mmHg)	HR (bpm)	eGFR	HbA1c (%)
1	F	66	N	Y	N	N	168	67	63	63.6	5.7
2	M	74	N	Y	N	N	164	70	63	46.2	5.5
3	M	72	Y	Y	N	N	166	95	78	63.8	6.1
4	F	92	Y	Y	N	N	162	65	54	68.5	6.2
5	F	68	N	N	N	N	179	87	65	91.2	5.4
6	M	74	N	Y	N	N	163	67	75	72.0	5
7	F	74	N	Y	N	N	178	82	71	61.6	4.9
8	M	82	Y	N	N	N	170	78	82	54.8	5.5
9	F	75	N	N	AP	N	168	57	70	26.6	5
10	M	66	Y	Y	N	N	182	84	68	101.9	5.8
11	M	82	Y	Y	AP	N	160	77	83	28.8	
12	F	72	N	N	N	N	160	83	93	62.1	5.4
13	M	73	N	Y	N	N	166	70	58	63.5	
14	F	54	N	N	N	N	170	103	89	79.8	5.4
15	F	76	N	Y	N	N	163	71	55	41.4	5.4
16	M	60	Y	Y	N	N	160	111	105	59.9	6.1
17	F	86	Y	N	N	N	187	69	64	39.9	6
18	F	77	N	Y	N	N	181	85	67	60.9	5.8
19	F	79	N	N	N	N	161	73	61	87.3	5.4
20	F	78	Y	Y	N	N	169	68	75	21.6	
21	M	81	Y	N	N	N	163	65	73		7
22	F	83	N	N	N	N	196	79	73	59.6	5.2
23	M	64	Y	Y	N	N	170	75	62	37.7	6.6
24	M	86	N	N	N	N	192	92	63	9.7	4.3
25	F	80	N	Y	AP	N	169	75	64	60.2	5.1
26	M	63	Y	Y	N	Y	173	78	68	14.1	

Treatment-resistant hypertension, R-HT for RD; M, male; F, female; DM, diabetes mellitus; Y, Yes; N, No; CHD, coronary heart disease; AP, angina pectoris; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); HbA1c, hemoglobin A1c.

ARB plus ACE-I (**Table 2**). All patients were taking CCB, including 3 taking 2 or 3 CCB. All were taking diuretics, including 5 patients using spironolactone. In addition, 8 patients were taking beta-blockers or alpha-blockers.

Eight patients had chronic kidney disease with an eGFR < 45 ml/min/1.73 m^2, accounting for 31% of the candidates for renal denervation.

The 65 patients with R-HT were divided into 2 groups, a renal denervation group (26 candidates for denervation) and a non-denervation group (the other 973 patients who were not candidates), for comparison by univariate

Table 2. Medication of R-HT for RD patients.

Patient	ARB	ACE-I name	CCB name	Diuretic	Sympathetic	Spironolactone	Vasodilator
1	Candesartan	Ｉmidapril	Amlodipine	Y	N	Y	N
2	Losartan		Cilnidipine	Y	N	N	N
3	Losartan		Benidipine	Y	N	N	N
4	Valsartan		Cilnidipine	Y	N	N	Y
5	Losartan		Nifedipine	Y	Y	N	N
6	Losartan	Ｉmidapril	Cilnidipine	Y	N	N	N
7	Valsartan		Amlodipine	Y	N	N	N
8	Valsartan, Losartan		Azelnidipine, Cilnidipine	Y	Y	Y	N
9	Valsartan		Nifedipine	Y	Y	N	Y
10	Losartan		Cilnidipine	Y	N	N	N
11	Ｉrbesartan, Losartan		Diltiazem	Y	N	N	N
12	Telmisartan		Benidipine	Y	N	N	N
13	Valsartan		Amlodipine	Y	N	N	N
14	Valsartan	Ｉmidapril	Amlodipine	Y	N	Y	N
15	Olmesartan	Lisinopril	Nifedipine	Y	N	N	N
16	Losartan		Nifedipine	Y	N	N	N
17	Olmesartan		Azelnidipine	Y	N	Y	N
18	Losartan		Amlodipine	Y	Y	N	N
19	Valsartan		Cilnidipine	Y	N	N	N
20	Olmesartan		Cilnidipine, Azelnidipine, Nifedipine	Y	Y	N	N
21	Valsartan		Nifedipine	Y	N	N	Y
22	Candesartan		Nifedipine	Y	N	N	N
23	Candesartan		Amlodipine, Nifedipine	Y	Y	Y	N
24	Ｉrbesartan		Diltiazem	Y	N	N	Y
25	Olmesartan		Diltiazem	Y	Y	N	Y
26		Enalapril	Nifedipine	Y	Y	N	N

ARB, angiotensin II receptor blocker; ACE-I, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker; For other abbreviations, see **Table 1**.

analysis (**Table 3**). The age, BNP, U-alb/U-cre ratio, baPWV, LVDd, and IVST were significantly higher/larger in the renal denervation group compared with the non-denervation group, while the levels of Hb, serum albumin, eGFR, PRA, PAC, and thyroxine were significantly lower. The U-Na/U-cre ratio tended to be higher in the renal denervation group. Complete data on HbA1c, eGFR, and BNP were not available for some patients, and they were excluded from the relevant univariate analyses.

In addition, we investigated independent factors related to patients being indicated for renal denervation by multivariate analysis, but we could not identify any significant factors.

Table 3. Comparison of the renal denervation (RD) group with the other patients (Non-RD group).

	RD group (N=26)		non-RD group (N = 973)		
	Frequencyy	Ratio (%)	Frequency	Ratio (%)	p value
Gender (Male)	12	46.2	512	52.7	NS
Smoking	1	3.9	156	16.0	NS
	n	Mean ± S.D	n	Mean ± S.D	p value
Age, years	26	74.5 ± 8.8	973	66.3 ± 13.5	<0.005
BMI, kg/m^2	26	24.5 ± 3.1	968	23.4 ± 3.5	NS
WBC, 10^3/μL	26	5.77 ± 1.37	969	5.50 ± 1.81	NS
Hb, g/dl	26	12.2 ± 2.2	969	13.4 ± 1.7	0.0001
PLT, 10^4/μL	26	20.1 ± 8.0	969	20.1 ± 5.5	NS
Alb, g/dL	24	4.0 ± 0.7	916	4.2 ± 0.4	<0.001
γ-GTP, U/L	26	26.9 ± 13.1	958	40.1 ± 71.4	NS
HbA1c, %	22	5.6 ± 0.6	913	5.6 ± 0.8	NS
eGFR, ml/min/1.73 m^2	25	55.1 ± 23.4	966	66.2 ± 19.1	<0.0005
UA, mg/dL	25	5.7 ± 1.6	959	5.4 ± 1.3	NS
HDL, mg/dL	25	65.5 ± 19.6	958	59.6 ± 14.5	0.09
LDL, mg/dL	25	104.1 ± 22.9	956	112.6 ± 28.1	NS
TG, mg/dL	25	111.8 ± 60.6	957	119.3 ± 75.2	NS
NEFA,μEq/L	20	565.6 ± 180.6	839	499.0 ± 234.7	0.095
CRP, mg/dL	24	0.1 ± 0.1	953	0.3 ± 1.2	NS
BNP, pg/mL	23	134.1 ± 254.6	861	47.9 ± 126.8	0.0001
baPWV, cm/sec	22	2079 ± 707	798	1790 ± 459	<0.01
LVDd, mm	24	50.7 ± 5.6	812	47.8 ± 4.8	<0.005
IVST, mm	24	9.3 ± 1.7	813	8.4 ± 1.4	<0.005
EF, %	24	66.8 ± 7.5	808	66.7 ± 7.9	NS
Spot U-alb/U-cre ratio*	24	417.9 ± 1072.9	902	78.1 ± 421.7	<0.005
Spot U-UA/U-cre ratio*	23	0.5 ± 0.2	878	0.5 ± 0.2	NS
Spot U-Na/U-cre ratio**	22	2.1 ± 1.4	860	1.7 ± 1.2	0.07
PRA, ng/ml/h	10	0.9 ± 0.8	292	1.5 ± 2.2	0.0001
PAC, pg/mL	10	38.2 ± 17.8	293	70.0 ± 61.0	<0.005
Adrenaline, pg/mL	9	42.1 ± 26.9	287	37.2 ± 29.4	NS
Noradrenalin, pg/mL	9	578.9 ± 551.0	287	384.8 ± 186.4	NS
Dopamine, pg/mL	9	18.2 ± 14.5	287	15.7 ± 48.7	NS
Cortisol, μg/dL	9	9.3 ± 3.4	286	10.1 ± 4.9	NS
TSH, μIU/mL	8	58.9 ± 161.7	321	2.1 ± 2.8	<0.05
FT4, ng//dL	8	1.1 ± 0.4	321	1.2 ± 0.2	<0.001

*(mg/dL)/(mg/dL); **(mmol/L)/(mg/dL); BMI, body mass index; WBC, White blood cells; Hb, hemoglobin; Plt, platelet count; alb, albumin; UA, uric acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; NEFA, non-esterified fatty acids; CRP, C-reactive protein; BNP, brain natriuretic peptide; baPWV, brachial-ankle pulse wave velocity; LVDd, left ventricular end-diastolic diameter; IVST, interventricular septal thickness; EF, ejection fraction; U, urinary; cre, creatinine; Na, sodium; PRA, plasma renin activity; PAC, plasma aldosterone concentration; TSH, thyroid stimulating hormone; FT4, free thyroxine; For other abbreviations, see **Table 1**.

4. Discussion

The major findings of the present study were as follows:

1) Among 999 outpatients with hypertension, 6.5% (n = 65) had R-HT and 2.6% (n =2 6) were candidates for renal sympathetic denervation (renal denervation group).

2) Compared with the 973 patients who were not indicated for denervation (non-denervation group), the renal denervation group was older, had a higher BNP, U-alb/U-cre ratio, U-Na/U-cre ratio, and baPWV, and had a higher LVDd and greater IVST on echocardiography. In addition, the renal denervation group had lower levels of Hb, albumin, eGFR, PRA, PAC, and thyroxine compared with the non-denervation group.

4.1. Characteristics of the Renal Denervation Group

According to the National Health and Nutrition Examination Survey conducted in the United States, 8.9% of adult patients with hypertension have R-HT and 12.8% of patients taking antihypertensive drugs have R-HT [5]. Compared with the United States, the prevalence of R-HT was lower (6.5%) in Japanese patients according to this study. Since it is known that salt intake is substantially higher among Japanese people than western populations [6], it is probable that diuretics are effective and reduce the prevalence of R-HT in Japan. Indeed, the lower PRA and PAC of our renal denervation group are characteristic of excessive salt intake [7]. The U-Na/U-cre ratio is a simple marker of salt intake used in clinical practice [8], and this ratio tended to be higher in our renal denervation group. Thus, it is likely that many patients in the renal denervation group had excessive salt intake.

Although there was a significant difference of thyroid hormone levels between the renal denervation group and the non-denervation group, 1 patient (No. 24) with thyroid dysfunction in the renal denervation group seemed to influence this result since the significant difference disappeared when data were analyzed after excluding the data of this patient. There were no significant differences of catecholamines between the 2 groups. In the renal denervation group, eGFR was significantly lower than in the non-denervation group and chronic kidney disease was more frequent (50%: 13/26 patients) than in the non-denervation group with (34%: 332/973 patients).

The high frequency of albuminuria and hypoalbuminemia in the renal denervation group may have been secondary to chronic kidney disease. The renal denervation group had a significantly lower Hb than the non-denervation group and showed a tendency for anemia, which could be related to lower erythropoietin production due to chronic kidney disease or hemodilution due to impaired excretion water resulting from renal dysfunction.

In the renal denervation group, patients tended to have left ventricular hypertrophy based on the echocardiography findings and BNP was also high. Thus, there was evidence of progressive cardiac remodeling associated with hypertension in this group.

Patients in the renal denervation group also showed a high baPWV, indicating advanced arterial stiffness. Therefore, despite treatment with multiple antihypertensive drugs, R-HT patients who were candidates for renal denervation had evidence of multiple organ impairment, a finding consistent with previous reports.

4.2. Investigating Candidates for Renal Denervation

A clinical trial of renal denervation was started in Japan, but it was halted in December 2014 due to difficulty in recruiting patients. One of the reasons for this outcome is that the indications for renal denervation are extremely limited [9]. In investigations of renal denervation, it is necessary to set limits on blood pressure and renal function, as well as excluding patients with secondary hypertension and masked-hypertension [10]. Because patients who require 3 antihypertensive drugs tend to have nephrosclerosis and renal dysfunction, many of them are unsuitable for renal denervation.

In the Symplicity HTN-3 study, patients between the ages of 18 and 80 were indicated for renal denervation [11]. In our study, 7 out of 26 candidate patients were at least 81 years old and thus were excluded. Another 3 patients (Nos. 18, 19, and 25) had reached the age of 81 at the time of performing this analysis, so they were also excluded, resulting in 10 patients (38%) being excluded because of their age.

Next, patients with chronic kidney disease and an eGFR < 45 ml/min/1.73 m^2 were excluded based on the published indications [11]. In this study, 8 of the candidates for renal denervation (31%) had an eGFR < 45 ml/min/1.73 m^2.

Taken together, the criteria for age and renal function excluded 15 of our candidate patients (58%) from renal

denervation, so these two factors seem to have a substantial influence on the number of patients who can actually undergo denervation.

The details of the remaining 11 patients are shown in **Table 4**. Before performing renal sympathetic denervation, it is necessary to exclude secondary causes of hypertension, such as renovascular hypertension, endocrine diseases including primary aldosteronism, Cushing syndrome, pheochromocytoma, hyperthyroidism, sleep apnea syndrome, and drug-induced hypertension [12].

In screening for primary aldosteronism, a PAC/PRA ratio >200 is usually employed [7] [13]. Two patients (Nos. 5 and 6) had a PAC/PRA ratio >200 and were suspected to have primary aldosteronism, so they were excluded from the renal denervation group for further assessment.

Another patient (No. 16) had a very high renin level that suggested renovascular hypertension and was also excluded (**Figure 1**).

There were no patients with suspected pheochromocytoma and no patients with pseudoaldosteronism caused by excessive consumption of licorice.

Since BP can be increased by salt intake, it is important to try lifestyle modification including control of salt intake before performing renal denervation. We evaluated salt intake from the U-Na and U-cre levels [8] [14], and identified 3 patients (Nos. 1, 12 and 13) who apparently had excessive salt intake. It was considered likely that salt restriction would improve the BP of these patients.

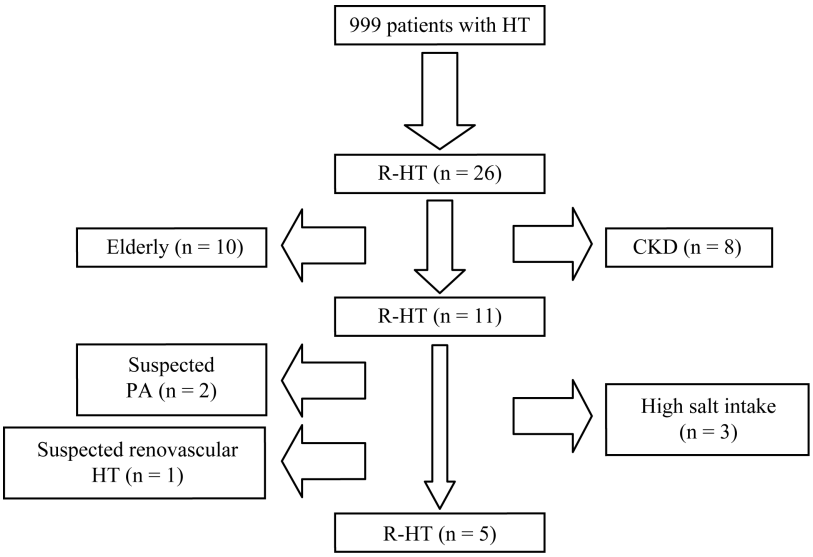
Finally, as shown in **Figure 1**, 5 out of 999 consecutive patients with hypertension were indicated for renal denervation, representing 0.5% of the subjects in our study. Of course, it is not clear that all of these patients would give consent to renal denervation and we also did not determine whether their renal artery anatomy was suitable, which would require further examination by renal arteriography, computed tomography, and ultrasound.

Thus, our study found very few hypertensive patients in whom renal denervation was indicated. In a similar study of 1756 hypertensive patients, only 14 patients (0.8%) were indicated for renal denervation [15], which is comparable to our rate of 0.5%. It has been reported that there are approximately 43 million hypertensive patients in Japan [16]. Since the prevalence of R-HT was 2.6% in our study, it can be estimated that there are approximately 1.12 million patients with R-HT in Japan, suggesting that renal denervation may be indicated in around 220,000 patients because the rate was 0.5% in the present study. Clinical trials of renal denervation in Japan would seem to have a problem with recruiting patients, but we first need to identify more patients with R-HT by screening and education. For example, establishing a large clinical cohort of hypertensive patients like

Table 4. Endocrine findings in R-HT candidates for renal denervation.

Patient	Gender	Age	BMI	PRA (ng/ml/h)	PAC (pg/ml)	ARR	U-Na (mEq/L)	U-Cre (mg/dL)	Salt intake (g/day)
1	F	66	32.8	0.4	66.3	165.8	76	10.8	16.3
2	M	74	27.1	0.3	36.2	120.7	92	86.6	8.9
3	M	72	24.3	0.4	39.7	99.3	106	167.2	7.6
5	F	68	21.1	0.2	104	520.0	49	50.6	6.9
6	M	74	25.7	0.2	71.3	356.5	166	132.6	9.1
7	F	74	20.0	0.7	65.5	93.6	158	73.4	9.5
10	M	66	22.8	2.5	51.7	20.7	87	56.8	10
12	F	72	22.4	1.5	38.9	25.9	121	17.3	16.7
13	M	73	29.2	0.5	42.6	85.2	172	96.9	12.5
14	F	54	23.1	2.2	118	53.6	87	290.4	4.7
16	M	60	22.9	>20	37	<1.85	97	608	<4.8

ARR, PAC/PRA ratio; For other abbreviations, see **Table 1** and **Table 3**.



Among 999 patients with hypertension (HT), there were 26 patients with resistant hypertension (R-HT) who were candidates for renal denervation. In this group, 10 patients were too old (>81 years) and 8 had chronic kidney disease (CKD). Because these patients overlapped, only 15 were excluded due to old age and CKD. The remaining 11 patients included 2 with suspected primary aldosteronism (PA), 1 with renovascular HT, and 3 with high salt intake. Finally, only 5 patients remained as candidates for renal denervation.

Figure 1. Selection of patients for renal denervation.

those at hypertension centers in Germany may help to identify R-HT patients by screening. Thus, we realized that not only for renal deneravation but for treatment of R-HT establishment of a large cohort including mild to severe hypertensive out patients was primary requirement.

It has been reported that renal denervation is more effective in patients with a higher BP before the procedure [17], suggesting that our patients No. 2 and No. 6 might be good candidates.

It was reported that Chinese are more sensitive than Caucasians to the effects of the beta-blocker propranolol on the pulse rate and BP [18]. Because Asians appear to be more sensitive to beta-blockers, renal denervation may be more effective for Asian patients with R-HT, and patients who have tachycardia like our No. 16 could be good candidates.

One cause of difficulty in controlling BP is poor adherence to medication, while good adherence can significantly improve control and reduce the risk of cardiovascular disease [19]-[21]. If a patient has to take multiple drugs, adherence is more likely to decrease, while reducing the frequency of medication or employing combination drugs can improve both adherence and BP [22] [23]. Thus, combining antihypertensive therapy and renal denervation may improve the prognosis of selected patients with hypertension.

4.3. Clinical Implications

After we started screening for renal denervation candidates at our hospital, we found more patients with secondary hypertension than before including cases of primary aldosteronism, renovascular hypertension, and pseudoaldosteronism due to licorice. This has led to an increase of adrenal venous sampling, renal arteriography, and arterioplasty at our hospital due to more comprehensive treatment of secondary hypertension. By results of this study, we realized the importance of diagnosis of secondary hypertension, intensive care by antihypertensive drugs and sodium restriction treatment.

Renal denervation will be recognized as a cost-effective intervention for hypertension if it improves life expectancy and other variables in outcome studies. In terms of surrogate endpoints, there is already evidence that renal denervation decreases arrhythmias such as atrial fibrillation, proteinuria, diabetes, sleep apnea syndrome, congestive heart failure, arteriosclerosis, and left ventricular remodeling [24]-[31]. More recently, it was reported that renal denervation improves depression and the quality of life [32] [33], while targeting suitable patients may contribute to reduction of medical expenses.

5. Limitations

Since this was a retrospective cross-sectional observational study, the findings are not conclusive. In addition, we did not employ ambulatory BP monitoring and only assessed the office BP, so it would be desirable to evaluate home BP and nocturnal BP in further studies. We also did not distinguish masked R-HT and sleep apnea syndrome from R-HT. Furthermore, we did not analyze adherence to medication or the doses of antihypertensive drugs used by the patients. Finally, complete data were not available for some patients and multivariate analysis failed to identify factors with an independent influence on R-HT.

6. Conclusion

In this retrospective analysis, we found that there were very few patients with R-HT among consecutive new outpatients with hypertension, and that renal denervation was indicated for only 0.5% of new hypertensive patients. Patients in whom renal denervation was indicated often had cardiomegaly and arteriosclerosis, and salt sensitivity was sometimes suspected as the underlying cause. Because many of the patients who may benefit from renal denervation have not been identified, it seems important to establish a cohort study that facilitates assessment of patients with R-HT.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Dahlof, B., Sever, P.S., Poulter, N.R., Wedel, H., Beevers, D.G., Caulfield, M., Collins, R., Kjeldsen, S.E., Kristinsson, A., McInnes, G.T., Mehlsen, J., Nieminen, M., O'Brien, E. and Ostergren, J. (2005) Prevention of Cardiovascular Events with an Antihypertensive Regimen of Amlodipine Adding Perindopril as Required versus Atenolol Adding Bendroflumethiazide as Required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): A Multicentre Randomised Controlled Trial. *The Lancet*, **366**, 895-906. http://dx.doi.org/10.1016/S0140-6736(05)67185-1

[2] Dahlof, B., Devereux, R.B., Kjeldsen, S.E., Julius, S., Beevers, G., de Faire, U., Fyhrquist, F., Ibsen, H., Kristiansson, K., Lederballe-Pedersen, O., Lindholm, L.H., Nieminen, M.S., Omvik, P., Oparil, S. and Wedel, H. (2002) Cardiovascular Morbidity and Mortality in the Losartan Intervention for Endpoint Reduction in Hypertension Study (LIFE): A Randomised Trial against Atenolol. *The Lancet*, **359**, 995-1003. http://dx.doi.org/10.1016/S0140-6736(02)08089-3

[3] Yusuf, S., Teo, K.K., Pogue, J., Dyal, L., Copland, I., Schumacher, H., Dagenais, G., Sleight, P. and Anderson, C. (2008) Telmisartan, Ramipril, or Both in Patients at High Risk for Vascular Events. *The New England Journal of Medicine*, **358**, 1547-1559. http://dx.doi.org/10.1056/NEJMoa0801317

[4] Matsuo, S., Imai, E., Horio, M., Yasuda, Y., Tomita, K., Nitta, K., Yamagata, K., Tomino, Y., Yokoyama, H. and Hishida, A. (2009) Revised Equations for Estimated GFR from Serum Creatinine in Japan. *American Journal of Kidney Diseases*, **53**, 982-992. http://dx.doi.org/10.1053/j.ajkd.2008.12.034

[5] Persell, S.D. (2011) Prevalence of Resistant Hypertension in the United States, 2003-2008. *Hypertension*, **57**, 1076-1080. http://dx.doi.org/10.1161/HYPERTENSIONAHA.111.170308

[6] The INTERSALT Co-Operative Research Group (1989) Appendix Tables. Centre-Specific Results by Age and Sex. *Journal of Human Hypertension*, **3**, 331-407.

[7] Funder, J.W., Carey, R.M., Fardella, C., Gomez-Sanchez, C.E., Mantero, F., Stowasser, M., Young Jr., W.F. and Montori, V.M. (2008) Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, **93**, 3266-3281. http://dx.doi.org/10.1210/jc.2008-0104

[8] Kawano, Y., Tsuchihashi, T., Matsuura, H., Ando, K., Fujita, T. and Ueshima, H. (2007) Report of the Working Group for Dietary Salt Reduction of the Japanese Society of Hypertension: (2) Assessment of Salt Intake in the Management of Hypertension. *Hypertension Research*, **30**, 887-893. http://dx.doi.org/10.1291/hypres.30.887

[9] Schlaich, M.P., Schmieder, R.E., Bakris, G., Blankestijn, P.J., Bohm, M., Campese, V.M., Francis, D.P., Grassi, G., Hering, D., Katholi, R., Kjeldsen, S., Krum, H., Mahfoud, F., Mancia, G., Messerli, F.H., Narkiewicz, K., Parati, G.,

Rocha-Singh, K.J., Ruilope, L.M., Rump, L.C., Sica, D.A., Sobotka, P.A., Tsioufis, C., Vonend, O., Weber, M.A., Williams, B., Zeller, T. and Esler, M.D. (2013) International Expert Consensus Statement: Percutaneous Transluminal Renal Denervation for the Treatment of Resistant Hypertension. *Journal of the American College of Cardiology*, **62**, 2031-2045. http://dx.doi.org/10.1016/j.jacc.2013.08.1616

[10] Esler, M.D., Krum, H., Sobotka, P.A., Schlaich, M.P., Schmieder, R.E. and Bohm, M. (2010) Renal Sympathetic Denervation in Patients with Treatment-Resistant Hypertension (The Symplicity HTN-2 Trial): A Randomised Controlled Trial. *The Lancet*, **376**, 1903-1909. http://dx.doi.org/10.1016/S0140-6736(10)62039-9

[11] Kandzari, D.E., Bhatt, D.L., Sobotka, P.A., O'Neill, W.W., Esler, M., Flack, J.M., Katzen, B.T., Leon, M.B., Massaro, J.M., Negoita, M., Oparil, S., Rocha-Singh, K., Straley, C., Townsend, R.R. and Bakris, G. (2012) Catheter-Based Renal Denervation for Resistant Hypertension: Rationale and Design of the SYMPLICITY HTN-3 Trial. *Clinical Cardiology*, **35**, 528-535. http://dx.doi.org/10.1002/clc.22008

[12] Calhoun, D.A., Jones, D., Textor, S., Goff, D.C., Murphy, T.P., Toto, R.D., White, A., Cushman, W.C., White, W., Sica, D., Ferdinand, K., Giles, T.D., Falkner, B. and Carey, R.M. (2008) Resistant Hypertension: Diagnosis, Evaluation, and Treatment. A Scientific Statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension*, **51**, 1403-1419. http://dx.doi.org/10.1161/HYPERTENSIONAHA.108.189141

[13] Nishizaka, M.K., Pratt-Ubunama, M., Zaman, M.A., Cofield, S. and Calhoun, D.A. (2005) Validity of Plasma Aldosterone-to-Renin Activity Ratio in African American and White Subjects with Resistant Hypertension. *American Journal of Hypertension*, **18**, 805-812. http://dx.doi.org/10.1016/j.amjhyper.2005.01.002

[14] Tanaka, T., Okamura, T., Miura, K., Kadowaki, T., Ueshima, H., Nakagawa, H. and Hashimoto, T. (2002) A Simple Method to Estimate Populational 24-h Urinary Sodium and Potassium Excretion Using a Casual Urine Specimen. *Journal of Human Hypertension*, **16**, 97-103. http://dx.doi.org/10.1038/sj.jhh.1001307

[15] Hayek, S.S., Abdou, M.H., Demoss, B.D., Legaspi, J.M., Veledar, E., Deka, A., Krishnan, S.K., Wilmot, K.A., Patel, A.D., Kumar, V.R. and Devireddy, C.M. (2013) Prevalence of Resistant Hypertension and Eligibility for Catheter-Based Renal Denervation in Hypertensive Outpatients. *American Journal of Hypertension*, **26**, 1452-1458. http://dx.doi.org/10.1093/ajh/hpt132

[16] Miura, K., Nagai, M. and Ohkubo, T. (2013) Epidemiology of Hypertension in Japan: Where Are We Now? *Circulation Journal*, **77**, 2226-2231. http://dx.doi.org/10.1253/circj.CJ-13-0847

[17] Prochnau, D., Heymel, S., Gobel, B., Figulla, H.R. and Surber, R. (2013) Resistant Hypertension: Multivariate Predictors of Blood Pressure Response to Renal Denervation. *International Journal of Cardiology*, **168**, 3130-3132. http://dx.doi.org/10.1016/j.ijcard.2013.04.035

[18] Zhou, H.H., Koshakji, R.P., Silberstein, D.J., Wilkinson, G.R. and Wood, A.J. (1989) Altered Sensitivity to and Clearance of Propranolol in Men of Chinese Descent as Compared with American Whites. *New England Journal of Medicine*, **320**, 565-570. http://dx.doi.org/10.1056/NEJM198903023200905

[19] Calhoun, D.A., Jones, D., Textor, S., Goff, D.C., Murphy, T.P., Toto, R.D., White, A., Cushman, W.C., White, W., Sica, D., Ferdinand, K., Giles, T.D., Falkner, B. and Carey, R.M. (2008) Resistant Hypertension: Diagnosis, Evaluation, and Treatment: A Scientific Statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation*, **117**, e510-e526. http://dx.doi.org/10.1161/CIRCULATIONAHA.108.189141

[20] Bramley, T.J., Gerbino, P.P., Nightengale, B.S. and Frech-Tamas, F. (2006) Relationship of Blood Pressure Control to Adherence with Antihypertensive Monotherapy in 13 Managed Care Organizations. *Journal of Managed Care Pharmacy*, **12**, 239-245.

[21] Corrao, G., Parodi, A., Nicotra, F., Zambon, A., Merlino, L., Cesana, G. and Mancia, G. (2011) Better Compliance to Antihypertensive Medications Reduces Cardiovascular Risk. *Journal of Hypertension*, **29**, 610-618. http://dx.doi.org/10.1097/HJH.0b013e328342ca97

[22] Claxton, A.J., Cramer, J. and Pierce, C. (2001) A Systematic Review of the Associations between Dose Regimens and Medication Compliance. *Clinical Therapeutics*, **23**, 1296-1310. http://dx.doi.org/10.1016/S0149-2918(01)80109-0

[23] Thom, S., Poulter, N., Field, J., Patel, A., Prabhakaran, D., Stanton, A., Grobbee, D.E., Bots, M.L., Reddy, K.S., Cidambi, R., Bompoint, S., Billot, L. and Rodgers, A. (2013) Effects of a Fixed-Dose Combination Strategy on Adherence and Risk Factors in Patients with or at High Risk of CVD: The UMPIRE Randomized Clinical Trial. *JAMA*, **310**, 918-929. http://dx.doi.org/10.1001/jama.2013.277064

[24] Dorenkamp, M., Bonaventura, K., Leber, A.W., Boldt, J., Sohns, C., Boldt, L.H., Haverkamp, W., Frei, U. and Roser, M. (2013) Potential Lifetime Cost-Effectiveness of Catheter-Based Renal Sympathetic Denervation in Patients with Resistant Hypertension. *European Heart Journal*, **34**, 451-461. http://dx.doi.org/10.1093/eurheartj/ehs355

[25] Geisler, B.P., Egan, B.M., Cohen, J.T., Garner, A.M., Akehurst, R.L., Esler, M.D. and Pietzsch, J.B. (2012) Cost-Effectiveness and Clinical Effectiveness of Catheter-Based Renal Denervation for Resistant Hypertension. *Journal of*

the American College of Cardiology, **60**, 1271-1277. http://dx.doi.org/10.1016/j.jacc.2012.07.029

[26] Pokushalov, E., Romanov, A., Corbucci, G., Artyomenko, S., Baranova, V., Turov, A., Shirokova, N., Karaskov, A., Mittal, S. and Steinberg, J.S. (2012) A Randomized Comparison of Pulmonary Vein Isolation with versus without Concomitant Renal Artery Denervation in Patients with Refractory Symptomatic Atrial Fibrillation and Resistant Hypertension. *Journal of the American College of Cardiology*, **60**, 1163-1170. http://dx.doi.org/10.1016/j.jacc.2012.05.036

[27] Mahfoud, F., Cremers, B., Janker, J., Link, B., Vonend, O., Ukena, C., Linz, D., Schmieder, R., Rump, L.C., Kindermann, I., Sobotka, P.A., Krum, H., Scheller, B., Schlaich, M., Laufs, U. and Bohm, M. (2012) Renal Hemodynamics and Renal Function after Catheter-Based Renal Sympathetic Denervation in Patients with Resistant Hypertension. *Hypertension*, **60**, 419-424. http://dx.doi.org/10.1161/HYPERTENSIONAHA.112.193870

[28] Mahfoud, F., Schlaich, M., Kindermann, I., Ukena, C., Cremers, B., Brandt, M.C., Hoppe, U.C., Vonend, O., Rump, L.C., Sobotka, P.A., Krum, H., Esler, M. and Bohm, M. (2011) Effect of Renal Sympathetic Denervation on Glucose Metabolism in Patients with Resistant Hypertension: A Pilot Study. *Circulation*, **123**, 1940-1946. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.991869

[29] Witkowski, A., Prejbisz, A., Florczak, E., Kadziela, J., Sliwinski, P., Bielen, P., Michalowska, I., Kabat, M., Warchol, E., Januszewicz, M., Narkiewicz, K., Somers, V.K., Sobotka, P.A. and Januszewicz, A. (2011) Effects of Renal Sympathetic Denervation on Blood Pressure, Sleep Apnea Course, and Glycemic Control in Patients with Resistant Hypertension and Sleep Apnea. *Hypertension*, **58**, 559-565. http://dx.doi.org/10.1161/HYPERTENSIONAHA.111.173799

[30] Hering, D., Lambert, E.A., Marusic, P., Ika-Sari, C., Walton, A.S., Krum, H., Sobotka, P.A., Mahfoud, F., Bohm, M., Lambert, G.W., Esler, M.D. and Schlaich, M.P. (2013) Renal Nerve Ablation Reduces Augmentation Index in Patients with Resistant Hypertension. *Journal of Hypertension*, **31**, 1893-1900. http://dx.doi.org/10.1097/HJH.0b013e3283622e58

[31] Brandt, M.C., Mahfoud, F., Reda, S., Schirmer, S.H., Erdmann, E., Bohm, M. and Hoppe, U.C. (2012) Renal Sympathetic Denervation Reduces Left Ventricular Hypertrophy and Improves Cardiac Function in Patients with Resistant Hypertension. *Journal of the American College of Cardiology*, **59**, 901-909. http://dx.doi.org/10.1016/j.jacc.2011.11.034

[32] Lenski, D., Kindermann, I., Lenski, M., Ukena, C., Bunz, M., Mahfoud, F. and Bohm, M. (2013) Anxiety, Depression, Quality of Life and Stress in Patients with Resistant Hypertension before and after Catheter-Based Renal Sympathetic Denervation. *EuroIntervention*, **9**, 700-708.

[33] Lambert, G.W., Hering, D., Esler, M.D., Marusic, P., Lambert, E.A., Tanamas, S.K., Shaw, J., Krum, H., Dixon, J.B., Barton, D.A. and Schlaich, M.P. (2012) Health-Related Quality of Life after Renal Denervation in Patients with Treatment-Resistant Hypertension. *Hypertension*, **60**, 1479-1484. http://dx.doi.org/10.1161/HYPERTENSIONAHA.112.200865

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The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*

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Abstract

Medicinal plants have played an essential role in the development of human culture. Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. This study illustrates the importance of traditional and modern medicines in the treatment and management of human diseases and ailments. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. Meanwhile, consumers in developed countries are becoming disillusioned with modern health care and are seeking alternatives. *Thymus vulgaris* is a species of flowering plant in the mint family Lamiacea. Thymus is a widely used medicinal plant in food and pharmaceutical industries. Among different species of Thymus, *Thymus vulgaris* is used more than other species in therapeutic dosage forms. In Traditional medicine *T. vulgaris* is cultivated in many countries by most people especially in rural areas depending on herbal medicines to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, insect bites, pains, etc. Also the modern medicine in essential oil of thyme has demonstrated that the compounds have shown anti-inflammatory, antioxidant, antibacterial and antifungal properties. In this review the objective is to consider the past and present value of medicinal plants such as *Thymus vulgar* is used in traditional and modern medical practices as bioactive natural compounds.

Keywords

Medicinal Plants, Traditional and Modern Medicine, *Thymus vulgaris*, Bioactive Compounds

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1. Introduction

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 - 5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India [1]. Medicinal plant is an important element of indigenous medical systems in all over the world. The ethno botany provides a rich resource for natural drug research and development [2]. "Traditional" use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as "traditional herbal medicines". In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons [3]. Natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates [4] and its importance in modern medicine has been discussed in different reviews and reports [5]. The value of natural products in this regard can be accessed from: 1) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification, 2) the number of diseases treated or prevented by these substances, and 3) their frequency of use in the treatment of disease. In recent years, the use of traditional medicine information on plant research has again received considerable interest [6]. In recent times, there have been increased waves of interest in the field of research in natural products chemistry. This level of interest can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products [7]. The World Health Organization (WHO) has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines [8]. Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. It is estimated that there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. In this review the objective is to consider the past and present value of medicinal plants such as *Thymus vulgaris* used in traditional and modern medical practices as bioactive natural compounds.

2. Plant Geographical Distribution and Its Description

Thymus vulgaris is a species of flowering plant in the mint family Lamiaceae, native to southern Europe from the western Mediterranean to southern Italy. It is a bushy, woody-based evergreen sub shrub with small, highly aromatic, grey-green leaves and clusters of purple or pink flowers in early summer [9]. Thyme grows well during a temperate to heat, dry, sunny climate, and wherever the plants don't seem to be shaded. Thyme species do best in coarse, rough soils that may be unsuitable for several alternative plants [10].

3. Background and History of Traditional Herbal Medicine

The use of plants as medicine goes back to early man. Fossil records date human use of plants as medicines at least to the middle Paleolithic age. Evidences of this early association have been found in the grave of a Neanderthal man buried 60,000 years ago. Pollen analysis indicated that the numerous plants buried with the corpse were all of medicinal value. The earliest known medical document is a 4000-year-old. Sumerian clay tablet that recorded plant remedies for various illnesses. By the time of the ancient Egyptian civilization, a great wealth of information already existed on medicinal plants. Among the many remedies prescribed were man drake for pain

relief, and garlic for the treatment of heart and circulatory disorders. This information, along with hundreds of other remedies, was preserved in the papyrus about 3500 years ago. Ancient China is also a source of information about the early medicinal uses of plants [11]. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12,000 items used by traditional healers, about 500 are uncommon use. Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market [12]. Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century [13]. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. One useful plant from this body of knowledge is snakeroot, used for centuries for its sedative effects. The number of higher plant species on this planet is estimated at 250,000, with a lower level at 215,000 [14] and an upper level as high as 500,000. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically. With high through put screening methods becoming more advanced and available, these numbers will change, but the primary discriminator in evaluating one plant species versus another is the matter of approach to finding leads. There are some broad starting points to selecting and obtaining plant material of potential therapeutic interest. However, the goals of such an endeavor are straight forward. Plants have an advantage in this area based on their long-term use by humans (often hundreds or thousands of years) [15].

4. Herbal Medicines in Developed Countries

Plants and their metabolites constituents have a long history of use in modern “western” medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, dioxin, morphine, quinine. Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century. In recent years, the use of traditional medicine information on plant research has again received considerable interest. While the western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources has become acknowledged by most academic and industrial researchers. Meanwhile, the need for basic scientific investigations on medicinal plants using indigenous medical systems becomes imminent [16]. The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines [17], particularly in Europe and North America, where herbal products have been incorporated into so-called alternative, “complementary”, “holistic” or “integrative” medical systems. Monographs on selected herbs are available from a number of sources, including the European Scientific Cooperative on Phytotherapy [18], German Commission E [19] and the World Health Organization [20]. The WHO monographs, for example, describe the herb itself by a number of criteria (including synonyms and vernacular names) and the herb part commonly used, its geographical distribution, tests used to identify and characterize the herb (including macroscopic and microscopic examination and purity testing), the active principles (when known), dosage forms and dosing, medicinal uses, pharmacology, contra-indications and adverse reactions. During the latter part of the twentieth century, increasing interest in self-care resulted in an enormous growth in popularity of traditional healing modalities, including the use of herbal remedies; this has been particularly true in the USA [21]. In the European market there are a lot of products derived from natural plants, which are recognized to possess different biological properties, such as antioxidant, antiseptic, diuretic, stimulating the central nervous system, sedative, expectorant, digestive, etc. Some of these plants have been used in traditional medicine since ancient times and are available on market as infusions, tablets and/or extracts. Consumers have reported positive attitudes towards these products, in large part because they believe them to be of “natural” rather than “synthetic” origin, they believe that such products are more likely to be safe than are drugs, they are considered part of a healthy lifestyle, and they can help to avoid un necessary contact with conventional “western” medicine [22].

5. Modern and Traditional Prescription of Herbal Drugs *(Thymus vulgaris)*

The pharmacological treatment of disease began long ago with the use of herbs. Although herbalist waned in the

eighteenth and nineteenth centuries, many of the remedies employed by the herbalists provided effective treatment. Some of these became useful prescriptions as physicians began experimenting with therapeutic agents. William Withering was the first in the medical field to scientific ally investigate a folk remedy. His studies (1775-1785) of foxglove as a treatment for dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the nineteenth century, scientists began purify the active extracts from medicinal plants. One breakthrough in pharmaceutical chemistry came when Friedrich isolated morphine from the opium poppy (*Papaver somniferum*) in 1806. Continuing this progress, Justus von Liebig, a German scientist became a leader in pioneering the field of pharmacology. With increased knowledge of active chemical ingredients, the first purely synthetic drugs based on natural products were formulated in the middle of the nineteenth century [22]. In plants, these compounds are mostly secondary metabolites under title bioactive compounds such as alkaloids, steroids, tannins, and phenol compounds, flavoneids which are capable of producing definite physiological action on body [23]. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in different ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day-to-day practice. Among all plant secondary metabolites which act as antioxidants phenol compounds form a large and varied group. Phenol compounds contribute significantly to the antioxidant potential of several plant species [24]. Among different Thymus species, *T. vulgaris* is cultivated in many countries by most people especially in rural areas depend on herbal medicines to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, insect bites, pains and so on [25]. *T. vulgaris* is used more in pharmaceutical dosage forms because it contains more essential oil than other species with high amount of thyme which exhibits considerable anti-tissue and expectorant effects [26]. Considering the fact that *Thymus vulgaris* is more expensive, some herbal industries tend to use other species of *Thymus* with different components. So it is crucial to develop a suitable and reliable identification method to confirm the quality of extracts and herbal drugs. Separation and detection of different constituents in plants have been always complicated. *Thymus* has approved expectorant, anti-tissue and antispasmodic activities. Its antiseptic property is estimated to be 25 times more effective than phenol, with less toxicity [27]. Different species of Thymus are different in content and type of components. Generally they contain thyme, carvacrol, flavonoids and phenol compounds such as rosmarinic acid which may have antiedemic and macrophage-inhibiting effects [28]. *Thymus vulgaris* shows a polymorphic variation in monoterpene production, the presence of intra-specific chemo type variation being common in the genus *Thymus*. Each of the six chemo types, geraniol (G), α-terpineol (A), thuyanol-4 (U), linalool (L), carvacrol (C), and thymol (T), is named after its dominant monoterpene [27]. Many pharmacological *in vitro* experiments carried out during the last decade revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma. Thymus vulgaris oil is a combination of monoterpenes and those will acts as anti-oxidative, antimicrobial, medicinal drug, anti-tissue, antispasmodic, and antibacterial activities [29].

6. Chemical Composition of the Essential oil of *Thymus vulgaris*

The essential oil from *T. vulgaris* showed a high content of oxygenated monoterpenes (56.53%) and low contents of monoterpene hydrocarbons (28.69%), sesquiterpene hydrocarbons (5.04%) and oxygenated sesquiterpenes (1.84%). The predominant compound among the essential oil components was thymol (51.34%) while the amount of all other components of the oil was less than 19% [30].

7. Medicinal Applications

Thyme has been thought of to be antiseptic, antimicrobial, medication, astringent, medicinal drug, carminative, disinfectant, medicinal drug and tonic. Thyme is incredibly useful in cases of assorted intestinal infections and infestations, like hook worms, gram-positive and gram-negative bacterium. Its active constituent, thymol, is active against coccid bacteria. Thyme may also improve liver functioning, and act as an appetite stimulant. It will be used in treatment of cartilaginous tube, bronchial and urinary infections [31]. Thyme is helpful in treatment

of laryngitis and inflammation. The main component of the volatile oil of thyme, thymol, is active against coccid bacteria. It is used for skin issues like oily skin, sciatica, acne, dermatitis, skin condition and bug bites. In aroma therapy, the distinct types, thymol, "red thyme oil", linalool kind for its terribly light soft action for antiviral properties are used. A corrected product, "white thyme oil" is also used, and it's milder on the skin. Applied to the skin, thyme relieves bites and stings, and relieves neuralgia and rheumatic aches and pains [32].

7.1. Anti-Oxidant Properties

An antioxidant is a molecule that inhibits the oxidation of different molecules. Oxidation is a chemical process that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions will produce free radicals. In turn, these radicals will begin chain reactions. Once the chain reaction happens in a cell, it will cause damage or death to the cell. Antioxidants stop these chain reactions by removing free radical intermediates, and inhibit different oxidation reactions. The leafy parts of *Thymus vulgaris* and its essential oil have been used in foods for the flavor, aroma and preservation and also in folk medicines. The components of *Thymus vulgaris* essential oil and the protective effects of this oil against aflatoxin-induce oxidative stress had been studied in some researches. The results indicated that the oil contains carvarcrol (45 mg/g), Thymol (24.7 mg/g), β-Phellandrene (9.7 mg/g), essential oil (4.1 mg/g), Humuline (3.1 mg/g), α-Phellandrene (2.3 mg/g) and Myrcene (2.1 mg/g) [33]. However, α and β-pinene, Myrcene, α-thyjone, Tricyclene, 1, 8-cineole, and β-sabinene were found in very lower concentrations. Treatment with aflatoxins alone disturbs lipid profile in blood serum, decreases total antioxidant capability, increase uric acid and nitric oxide in blood serum and lipid per-oxidation in liver and excretory organ attended with a sever histological changes within the liver tissues. The oil alone at the 2 tested doses didn't induce any important changes within the biochemical parameters or the histological image. The combined treatment showed important enhancements altogether tested parameters and histological foot age within the liver tissues. Moreover, this improvement was additional pronounced within the cluster received the high dose of the oil [34].

7.2. Anti-Bacterial Activity

The essential oils obtained from *Thymus vulgaris* harvested at 4 biological process stages were evaluated for their biological activity and chemical components. The thyme volatile oils were analyzed for their inhibition effects against 9 strains of gram-negative bacteria and 6 strains of gram-positive bacteria. The bio-impedance methodology was chosen for finding out the antibacterial activity of the essential oils and also the parameter chosen for outlining and quantifying the antibacterial activity of the thyme oils was the detection time. The plate counting technique was used to studying the inhibitory effect by direct exposure. All the thyme essential oils examined had significant bacteria static activity against the microorganisms tested. This activity was additional marked against the gram-positive bacteria. The oil from thyme fully flower was the most effective at stopping the growth of the microorganism species examined. The oils tested were conjointly shown to possess smart antibacterial activity by direct contact, that gave the impression to be a lot of marked against the gram-negative microorganism. Some a number of the species were capable of recovering a minimum of 50% of their metabolic function once contact with the inhibitor, whereas most of the strains were shown to have been inactivated almost completely [35].

7.3. Anti-Viral Property

Silke Nolkemper *et al*. conducted an experiment with aqueous extracts from species of the Lamiaceae family were examined for their antiviral activity against Herpes simplex virus (HSV). Extracts from thyme (*Thymus vulgaris*) has shown inhibitory activity against Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and an acyclovir-resistant strain of HSV-1 was tested in vitro on RC-37 cells in a plaque reduction assay [36].

7.4. Anti-Inflammatory Property

Thymus vulgaris oil is a combination of monoterpenes. The most compounds of this oil are the natural terpenoid thymol and its phenol chemical compound carvacrol [34] that has medicinal drug, anti-oxidative, antimicrobial, anti-tissue, antispasmodic, and antibacterial effects [16]. Terpenoids, flavonoide glycosides, and synthetic resin acids were additionally found in Thymus spp.

7.5. Insecticidal Activity

The insecticidal activity of thyme volatile oil, thymol and carvacrol was evaluated in laboratory against completely different larval stages of lesser meal worm. The sooner and later larval stages were reared on diets containing one or two acetone solutions of tested compounds. Insecticidal activity of thyme volatile oil and pure monoterpenes against larvae relied on the dose and age of larvae. The growth of younger larvae was considerably affected, whereas those of older larval stage were less influenced and only by pure oil components. In young larvae the application 1% thyme oil, thymol and carvacrol, caused mortality of 50.0%, 86.67% and 85%, respectively [37].

8. Conclusion

While centuries of use in traditional settings can be used as testimony that a particular herbal ingredient is effective or safe, several problems must be addressed as these ingredients are incorporated into modern practice. One problem is that ingredients once used for symptomatic management in traditional healing are now used in developed countries as part of health promotion or disease prevention strategies; thus, acute treatment has been replaced by chronic exposure. This means that a statement about "thousands of years of evidence that a product is safe" may not be valid for the way the product is now being used. This does not expressly mean that an ingredient is unsafe; it does mean that safety in the modern context cannot be assumed. A second problem is that efficacy and effectiveness have rarely been demonstrated using modern scientific investigations. An evidence-based approach to this issue has only recently been implemented, and the results reveal that for most herbal products, considerable gaps in knowledge need to be remedied before one can be convinced about their efficacy. Is traditional medicine reality essential in the world? The more salient problems associated with "*modern*" medicine are as follows: 1) Its indiscriminative and aggressive dispossession of local health and medical knowledge accumulated by trial and error over many centuries and in some cases millennia; 2) its systematic displacement of the potential for genuine local control and familial participation in the key processes of prevention and cure; 3) it's admitted in capacity to reverse some infectious, and all degenerative and autoimmune diseases; and 4) its swelling wake of (largely unmonitored and uncompensated) problems due to its chosen methods of prevention and cure. The outset of the new millennium the World Health Organization (WHO) estimated that the majority of the populations of most countries were still relying primarily upon indigenous or traditional forms of medicine for meeting every day health care needs. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Consumers have reported positive attitudes towards these products, in large part because they believe them to be of "natural" rather than "synthetic" origin, they believe that such products are likely to be more safe than drugs, they are considered part of a healthy life style, and they can help to avoid unnecessary contact with conventional "western" medicine. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons.

References

[1] Prakash, P. and Gupta, N. (2005) Therapeutic Uses of *Ocimum sanctum* Linn (Tulsi) with a Note on Eugenol and Its Pharmacological Actions: A Review. *Indian Journal of Physiology and Pharmacology*, **49**, 125-131.

[2] Farnsworth, N.R. (1990) The Role of Ethno Pharmacology in Drug Development. Ciba Foundation Symposium 154. Bioactive Compounds from Plants. John Wiley & Sons, Baffins Lane, Chichester (England), 2-21.

[3] Vishwakarma, A.P., Vishwe, A., Sahu, P. and Chaurasiya, A. (2013) Magical Remedies of *Terminalia arjuna* (ROXB.). *International Journal of Pharmaceutical Archive*, **2**, 189-201.

[4] Newman, D.J., Crag, G.M. and Snader, K.M. (2000) The Influence of Natural Products upon Drug Discovery. *Natural Product Reports*, **17**, 215-234. http://dx.doi.org/10.1039/a902202c

[5] Jones, W.P., Chin, Y.-W. and Kinghorn, A.D. (2006) The Role of Pharmachology in Modern Medicine and Pharmacy. *Current Drug Targets*, **7**, 247-264. http://dx.doi.org/10.2174/138945006776054915

[6] Newman, D.J. and Crag, G.M. (2007) Natural Products as Sources of New Drugs over the Last 25 Years. *Journal of Natural Products*, **70**, 461-477. http://dx.doi.org/10.1021/np068054v

[7] Clark, A.M. (1996) Natural Products as a Source for New Drugs. *Pharmaceutical Research*, **13**, 1133-1141. http://dx.doi.org/10.1023/A:1016091631721

[8] WHO (1993) Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila.

[9] Christopher, B. (2008) RHS A-Z Encyclopedia of Garden Plants. Dorling Kindersley, United Kingdom.

[10] http://bioweb.uwlax.edu/bio203/s2012/disrud_sama/classification.htm

[11] Xiao, P. (1988) A Pictorial Encyclopaedia of Chinese Medicine. v1-10. Commercial Press, Hong Kong, 88-90.

[12] Li, L. (2000) Opportunity and Challenge of Traditional Chinese Medicine in Face of the Entrance to WTO (World Trade Organization). *Chinese Journal of Information on Traditional Chinese Medicine*, **7**, 7-8.

[13] Saito, H. (2000) Regulation of Herbal Medicines in Japan. *Pharmacological Research*, **41**, 515-519. http://dx.doi.org/10.1006/phrs.1999.0645

[14] Arash, R., Koshy, P. and Sekaran, M. (2010) Antioxidant Potential and Phenol Content of Ethanol Extract of Selected Malaysian Plants. *Research Journal of Biotechnology*, **5**, 16-19.

[15] Tippo, O. and Stern, W.L. (1977) Humanistic Botany. W.W. Norton, New York.

[16] Taylor, L. (2000) Plant Based Drugs and Medicines. Raintree Nutrition Inc., Carson City, NV.

[17] Tyler, V.E. (1999) Phytomedicines: Back to the Future. *Journal of Natural Products*, **62**, 1589-1592. http://dx.doi.org/10.1021/np9904049

[18] ESCOP (European Scientific Cooperative on Phytotherapy) (1999) ESCOP Monographs on the Medicinal Uses of Plant Drugs, Exeter, UK.

[19] Blumenthal, M., Busse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C.W. and Rister, R.S. (1998) The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines, Austin, TX/Boston, MA, American Botanical Council/Integrative Medicine Communications.

[20] WHO (1999) WHO Monographs on Selected Medicinal Plants, Vol. 1, Geneva.

[21] Fumonisin, B. (2002) Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 82.

[22] Schulz, V., Hänsel, R. and Tyler, V.E. (2001) Rational Phytotherapy. A Physician's Guide to Herbal Medicine. 4th Edition, Springer-Verlag, Berlin. http://dx.doi.org/10.1007/978-3-642-98093-0

[23] Donald, P.B. (2000) Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. *Plant Physiology*, **124**, 507-514. http://dx.doi.org/10.1104/pp.124.2.507

[24] Cronquist, A. (1988) The Evolution and Classification of Flowering Plants. New York Botanical Garden, Bronx.

[25] Namsa, N.D., Tag, H., Mandal, M., Kalita, P. and Das, A.K. (2009) An Ethnobotanical Study of Traditional Anti-Inflammatory Plants Used by the Lohit Community of Arunachal Pradesh, India. *Journal of Ethnopharmacology*, **125**, 234-245. http://dx.doi.org/10.1016/j.jep.2009.07.004

[26] Verpoorte, R. (2000) Pharmacognosy in the New Millennium: Leadfinding and Biotechnology. *Journal of Pharmacy and Pharmacology*, **52**, 253-262. http://dx.doi.org/10.1211/0022357001773931

[27] Thompson, J., Chalcha, J., Michet, A., Linhart, Y. and Ehlers, B. (2003) Qualitative and Quantitative Variation in Monoterpene Co-Occurrence and Composition in the Essential Oil of *Thymus vulgaris* Chemotypes. *Journal of Chemical Ecology*, **29**, 859-880. http://dx.doi.org/10.1023/A:1022927615442

[28] Zarzuelo, A. and Crespo, E. (2002) The Medicinal and Non Medicinal Uses of Thyme. *Thyme*: *The Genus Thymus*, 263-292.

[29] Marculescu, A., Vlase, L., Hanganu, D., Dragulescu, C., Antonie, I. and Neli-Kinga, O. (2007) Polyphenols Analyses from *Thymus* Species. *Proceedings of the Romanian Academy—Series B*, **3**, 117-121.

[30] Maher Ali Ahmed, M., Saeed Mohammed, A. and Ebtesam Hasan, A. (2011) Chemical Composition and Antimicrobial Activity of Essential Oil of *Thymus vulgaris* from Yemen. *Turkish Journal of Biochemistry*, **36**, 342-349.

[31] Amiri, H. (2012) Essential Oils Composition and Antioxidant Properties of Three *Thymus* Species. *Evidence-Based Complementary and Alternative Medicine*, **2012**, Article ID: 728065. http://dx.doi.org/10.1155/2012/728065

[32] ESCOP (2007) ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products. The European Scientific Cooperative on Phytotherapy in Collaboration with Georg Thieme.

[33] El-Nekeety, A.A., Mohamed, S.R., Hathout, A.S., Hassan, N.S., Aly, S.E. and Abdel-Wahhab, M.A. (2011) Antioxidant Properties of *Thymus vulgaris* Oil against Aflatoxin-Induce Oxidative Stress in Male Rats. *Toxicon*, **57**, 984-991. http://dx.doi.org/10.1016/j.toxicon.2011.03.021

[34] Nickavar, B., Mojab, F. and Dolat-Abadi, R. (2005) Analysis of the Essential Oils of two *Thymus* Species from Iran. *Food Chemistry*, **90**, 609-611. http://dx.doi.org/10.1016/j.foodchem.2004.04.020

[35] Marino, M. and Bersani, C. (1999) Antimicrobial Activity of the Essential Oils of *Thymus vulgaris* L. Measured Using a Bioimpedometric Method. *Journal of Food Protection*, **62**, 1017-1023.

[36] Nolkemper, S., Reichling, J., Stintzing, F.C., Carle, R. and Schnitzler, P. (2006) Antiviral Effect of Aqueous Extracts from Species of the Lamiaceae Family against *Herpes simplex* Virus Type 1 and Type 2 *in Vitro*. *Planta Medica*, **72**, 1378-1382. http://dx.doi.org/10.1055/s-2006-951719

[37] Szczepanik, M., Zawitowska, B. and Szumny, A. (2012) Insecticidal Activities of *Thymus vulgaris* Essential Oil and Its Components (Thymol and Carvacrol) against Larvae of Lesser Mealworm, Alphitobiusdiaperinus Panzer (Coleoptera: Tenebrionidae). *Allelopathy Journal*, **30**, 129-142.

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Isolated Tracheobronchial Amyloidosis

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Abstract

The tracheobronchial amyloidosis is a rarely localized form. It may manifest as a cancer. Only histological results allow diagnosis confirmation. A 68-year-old man was admitted for exploration of a low abundance hemoptysis. The chest radiograph was normal. CT scan objectified thickening of the walls of the trachea and bronchi which are the seat of calcifications. Bronchoscopy found a burgeoning infiltrative lesion training the right side face of the trachea and the anterior edge of the hull. Histological results confirmed tracheobronchial amyloidosis. Typing of this amyloidosis concluded to AL form. The study of amyloid extension lesion was negative. Immunoelectrophoresis of proteins in the blood and urine was normal. The patient was sent to receive external beam radiation to prepare for endoscopic surgical removal. Post-operative suites were marked by a complete resolution of clinical signs particularly hemoptysis with a follow-up of two years.

Keywords

Amyloidosis, Trachea, Biopsy

1. Introduction

Amyloidosis is the extracellular deposition of amyloid substance having specific structure. Localized respiratory form of amyloidosis is extremely rare, particularly tracheobronchial site [1] [2].

Amyloidosis is not often taken into consideration in the differential diagnosis of patients with respiratory symptoms.

No clinical, biological and radiological feature is specific of the tracheobronchial localization [1]-[3].

Histological examination of a biopsy specimen guided by bronchoscopy confirms the diagnosis.

The immunohistochemical analysis allows for the typing of amyloidosis (AA, AL, ...) [1] [2].

The aim of this work is to review the scarcity of tracheobronchial amyloidosis, the diagnostic difficulty and the need for further monitoring because of the risk of transition to the systemic form and occurrence of

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life-threatening complications.

2-Case Report

A 68-year-old man, with a history of a large smoking, had consulted for a low abundance hemoptysis.

The physical examination was without abnormalities particularly cardiac and pulmonary auscultation as well as neurological and abdominal examinations.

Biological investigations did not reveal anemia or inflammatory syndrome. The white blood cells count, the platelets count and the hemostasis tests were normal. The proteinuria was negative.

The chest radiograph was without anomalies.

Chest CT scan objectified thickening of the walls of the trachea with a moderate luminal narrowing and bronchi which are the seat of calcifications. Neither lymph node enlargement nor pleural effusion was found.

Bronchoscopy found a burgeoning infiltrative lesion training the right side face of the trachea and the anterior edge of the hull.

The hitological examination of tracheobronchial biopsy found an amyloidosis. Typing of this amyloidosis concluded to AL form.

The patient had no other visceral localization of amyloidosis implicating heart, kidney, bvgusalivary glands and bone marrow.

Immunoelectrophoresis of proteins in the blood and urine was normal.

The patient was sent to receive external beam radiation to prepare for endoscopic surgical removal.

Post-operative suites were marked by a complete resolution of clinical signs particularly hemoptysis with a follow-up of two years.

3. Discussion

The tracheobronchial amyloidosis is rare; about a hundred cases have been reported in the literature [2]-[4]. This is a localized form of amyloidosis [2] [3]. The most common amyloid pulmonary manifestations are tracheobronchial, nodular, and diffuse interstitial infiltrations.

It is usually symptomatically mimicking at many points bronchial neoplasia particularly when it occurs in a large smoking patient. It most frequently presents by coughing, wheezing, hemoptysis, or unexplained and recurrent pneumopathy [2]-[4].

The diagnosis is oriented by chest CT and confirmed by biopsy during bronchoscopy [2]-[5].

Neither chest CT results nor macroscopic aspects at bronchoscopy show specific signs.

The thickening aspect of the tracheal walls revealed in our case can be encountered in many diagnoses as carcinoma, lymphoma and sarcoidosis.

The clinical, radiological and endoscopic feature isn't contributive for diagnosis confirmation.

Only histological results showing amyloid deposits in the biopsied tissues allow confirming the diagnosis. Specific colorations are recommended to avoid missing the diagnosis.

Solitary nodules or elongated airway narrowing may be mistaken for neoplasia [6].

After confirming the diagnosis, investigations guided by the symptoms are necessary in search of systemic amyloidosis; cardiac and renal ultrasound evaluations are required to evaluate the patient prognosis [2]. Sometimes, many latent amyloid localizations can be revealed in the occasion of systematic research of other systemic involvement

In our case, tracheo-bronchial amyloidosis was isolated.

A bone marrow aspirate, Immunoelectrophoresis of proteins in the blood and urine are essential, in search of multiple myeloma.

Annual monitoring during the first ten years is essential since there's a possibility of conversion into systemic disease whose treatment differs from the localized form, almost progression to severe and permanent respiratory impairment and metastatic transformation may occur [6].

Rare similar cases to our patient were reported in the literature.

Brill AK *et al*. reported the case of a 65-year-old woman presenting recurrent bronchopulmonary infections, permanent cough and progressive dyspnea. The diagnosis of tracheobronchial amyloidosis was made by histological findings [6].

Yap JC presented a case of isolated tracheobronchial amyloidosis in a 72-year-old woman complaining of

recurrent cough, dyspnoea, haemoptysis and respiratory infection. She received laser therapy with a good evolution [7].

The treatment of the localized tracheobronchial form is symptomatic.

The treatments offered in the localized tracheobronchial amyloidosis include external beam radiation, endoscopic procedures, debridement, removal and laser [6] [7].

Elongated stenosis can be managed by laser therapy, balloon dilatation or stenting.

Surgical treatment is rarely indicated. In case of localized AL (amyloid light chain) radiotherapy can be proposed in AL form [6].

Spontaneous regression has also been noted [8].

Bronchoscopic procedures and slow progression of this localized amyloidosis allow prognosis improvement. The prognosis is rarely worsened by the evolution to respiratory failure [2]-[4] [6] [8].

4. Conclusion

Localized amyloidosis such as tracheobronchial location, is rare and may be the first sign of a systemic disease, the pathogenesis remains unclear. Possible respiratory and systemic complications indicate a regular and prolonged surveillance.

Disclosure Statement

The authors have nothing to disclose.

References

[1] Georgiades, C.S., Neyman, E.G., Barish, M.A. and Fishman, E.K. (2004) Amyloidosis. A Review and CT Manifestations. *Radiographics*, **24**, 405-416. http://dx.doi.org/10.1148/rg.242035114

[2] Cazalets, C., Belleguic, C., Sost, G., Caulet-Maugendre, S., Kernec, J., Droz, D. and Grosbois, B. (2002) Tracheo-Bronchial Amyloidosis: Report of Two Cases. *La Revue de Médecine Interne*, **23**, 317-321. http://dx.doi.org/10.1016/S0248-8663(01)00557-4

[3] Cazalets, C., Cadora, B., Mauduitb, N., Decaux, O., Ramée, M.-P., Le Pogamp, P., *et al.* (2003) Epidemiologic Description of Amyloidosis Diagnosed in University Hospital of Rennes from 1995 to 1999. *La Revue de Médecine Interne*, **24**, 424-430. http://dx.doi.org/10.1016/S0248-8663(03)00136-X

[4] Racil, H., Ben Amar, J., Rouhou, S.C., Laaribi, G., Ayadi, A., Zarrouk, M., Chaouch, N. and Chabbou, A. (2011) Haemoptysis Revealing Tracheobronchial Amyloidosis. *Revue de Pneumologie Clinique*, **67**, 109-112. http://dx.doi.org/10.1016/j.pneumo.2010.02.003

[5] Jiang, X. and Wang, G. (2012) On Primary Pulmonary Amyloidosis. *European Journal of Radiology*, **81**, 1968. http://dx.doi.org/10.1016/j.ejrad.2011.04.005

[6] Brill, A.K., Woelke, K., Schädlich, R., Weinz, C. and Laier-Groeneveld, G. (2007) Tracheobronchial Amyloidosis—Bronchoscopic Diagnosis and Therapy of an Uncommon Disease: A Case Report. *Journal of Physiology and Pharmacology*, **58**, 51-55.

[7] Yap, J.C., Wang, Y.T. and Poh, S.C. (1992) A Case of Primary Diffuse Tracheobronchial Amyloidosis Treated by Laser Therapy. *Singapore Medical Journal*, **33**, 198-200.

[8] Hop, D.G. and Rasp, F.L. (1979) Spontaneous Regression of Tracheobronchial Amyloidosis. *Chest*, **76**, 237-239.

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Stenosis of the Subglottic Trachea Revealing Relapsing Polychondritis of a Young Adult

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Abstract

Relapsing polychondritis is a rare inflammatory disease involving essentially cartilaginous structures. Other systemic manifestations can be encountered as the eye and ear disturbance. Aortic aneurysms affect few cases. If affection of airway cartilages occurs, prognosis may be worsened by eventual stenotic lesions. We report a 22-year-old woman with Relapsing polychondritis. When she was referred to our hospital 4 weeks after the onset of respiratory symptoms, she was having severe breathing difficulty. Immediate tracheostomy followed by steroid therapy improved her respiratory condition, although the treatment was complicated because of her diabetes. While airway involvement of Relapsing polychondritis can be life threatening, it is curable with steroid therapy. Clinicians should keep in mind that airway obstruction could be caused by this disease.

Keywords

Relapsing Polychondritis, Airway Narrowing, Prognosis

1. Introduction

Relapsing polychondritis (RP) is a disease of unknown etiology characterized by recurrent non-infectious inflammation of cartilaginous and connective tissues. It is an uncommon, chronic, and potentially life-threatening multisystem disorder in case of laryngeal and tracheobronchial disturbance. The etiology of RP is still unknown, but the pathogenetic role of the autoimmunity is suggested by frequent overlaps with various autoimmune diseases, and by the presence of autoantibody against cartilage in the serum of patients with RP [1] [2].

Clinical presentations of RP vary considerably from patient to patient, and the involvement of multiple organs has been reported. Although several reports have demonstrated the clinical signs, pathologic manifestations and

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radiologic findings of RP, there are no specific features of RP. Therefore, it is difficult to show the diagnosis of RP [1] [2].

Although several reports have demonstrated the clinical signs, pathologic manifestations and radiologic findings of RP, there are no specific features of RP.

Therefore, it is difficult to show the diagnosis of RP. RP is clinically diagnosed; there are no specific biological or radiological signs to confirm the diagnosis.

Radiographic study of the airway is of great value in detection and evaluation of upper respiratory involvement. Airway involvements are major causes of morbidity and mortality, and they have accounted for most of the deaths due to RP. To suppress the inflammation of airway mucosa and cartilage is extremely important in the successful treatment for RP.

RP is a polymorph multisystem pathology with various manifestations, inducing thus serious and refractory damage such as trachea stenosis due to lateness in the diagnostic delay and therapeutic management. Only rapid diagnosis and efficient therapy are guarantors of improving the prognosis and avoiding sequelae.

The aim of this study is to show that RP diagnosis should be considered in case of airway narrowing. Only an early diagnosis and an adapted treatment may improve prognosis.

2. Observation

A 27-year-old woman presented with eight-months history of fever, productive cough, and dyspnea. A radiographic lateral view of the neck showed subglottic tracheal stenosis. A computed tomography (CT) scan of the neck also showed narrowing of the larynx (approximately 3 cm until the carena). An arterial blood gas test showed a pH: 7.4, $PaCO_2$: 37 mmHg, PaO_2: 60 mmHg, SaO_2: 94%, and HCO_3: 24 mEq/L in room air. The CRP rate was 62 mg/L and ferritin was at 560 ng/L. Other laboratory findings were in the normal range. The laryngeal biopsy showed a non-specific catarrhal laryngitis Computed tomography (CT) depicted diffuse bronchotracheal stenosis, which deteriorated in exhalation. The MRI revealed airway stenosis from the trachea to the lobar bronchi. She was treated with glucocorticoid without success and the intubation was indicated. Owing to potential risks of airway compromise during intubation and/or extubation and the regression of tracheal edema, she was extubated 5 days after. Tracheotomy was made with Shiley canule. Few months later, she developed bilateral anterior uveitis and left auricular chondritis treated with glucocorticoid with success. In conjunction with nasal, which was previously overlooked, RP was finally diagnosed We made a diagnosis of relapsing polychondritis (RP) based on the following criteria set by MacAdam *et al.* and Damiani and Levine: 1) bilateral auricular chondritis; 2) nonerosive seronegative inflammatory polyarthritis; 3) nasal chondritis; 4) ocular inflammation; 5) respiratory tract chondritis; and 6) audiovestibular damage. Administration of high-dose glucocorticoid and azathioprine resolved the symptoms and the bronchial stenosis was stabilized. She developed 6 months later diabetes treated with metformine.

3. Discussion

RP is a relapsing degenerative disease of cartilaginous tissues characterized by the involvement of multiple organs and vessels, including the nose, ears, throat, trachea, eyes, joints and cardiac valves; however, the etiology of RP is still not fully understood [1]-[3].

RP is a Th1-mediated disease marked by high serum levels of interferon γ, interleukin 12 and interleukin 2. In addition, serum levels of vascular endothelial growth factor and matrix metalloproteinases-3 are high in RP and are correlated with disease activity. Auto-immune reaction can be induced by infectious

Mechanical and chemical aggressions implicating cartilaginous structure may induce the occurrence of RP. Genetic Susceptibility to RP has been also reported.

Previous studies have suggested that autoimmunity may be associated with the pathogenesis of RP. No ethnicity, sex or age differences have been reported for RP, but most patients with RP are between 40 and 60 years of age [1] [2]. Currently, most medical practitioners and researchers apply the criteria proposed by Damiani *et al.* [3] for the diagnosis of RP. As RP is a rare disease with clinical manifestations that vary from patient to patient, it is very hard to diagnose, especially in patients with atypical symptoms. The early diagnosis of RP is even more challenging [1]. Laboratory investigations in this case revealed increased CRP, in accordance with previous studies [4]; interestingly, we also found an increased ferritin level, consistent with the findings of Fujiki *et al.* [5].

Airway involvement by RP is generally considered ominous and has been reported to portend a poor prognosis [6] [7]. Tracheobronchomalacia (TBM), due to loss of the supportive cartilaginous scaffolding of the upper respiratory airways, can be seen as chronic sequelae of RP due to recurrent inflammation [8]. TBM may also be the sole airway abnormality recognized during the early stages of RP. Respiratory compromise stemming from fixed airway obstruction or hyperdynamic collapse may cause significant morbidity and mortality [9]. Despite aggressive medical therapy, many patients experience symptom progression and eventually require tracheotomy, which may not be fully palliative due to frequent obstruction and malacia of more distal airways beyond the tracheotomy site [3] [10].

Lower airway manifestations of RP can be the only sign of the disease. RP has to be considered in the differential diagnosis of patients with recent onset of progressive dyspnea and severe airflow limitation even without other systemic signs of cartilage damage [9].

Unfortunately, our knowledge about airway involvement in RP is limited and is based on anecdotal reports and small case series. Purcelli *et al.* [11] described the first case report of RP with tracheal collapse in 1962, and McAdam *et al*. [8] reported in a case series in 1976 that the majority of patients with RP and a known cause of death had respiratory tract involvement. In many of those patients, airway problems were in fact the complaints leading to the diagnosis of RP, underscoring the fact that clinically significant airway compromise does not necessarily present late in the course of the disease. The majority of patients with respiratory symptoms who were referred for airway evaluation were women (70%) like our case, a finding that differs from those in some reports that have described an equal gender distribution in patients with a clinical diagnosis of RP as a whole but is similar to those in other reports in the literature [10].

RP is a disorder that has a variety of clinical manifestations and is considered an autoimmune disease resulting in cartilage breakdown. In this case, the patient demonstrated severe airway stenosis on CT that was well correlated with her severe respiratory symptoms. The most common causes of death among patients with RP are infection, airway compromise, and cardiac complications [12].

Symptomatic tracheobronchial involvement implies a poor prognosis. Laryngotracheal symptoms are present in approximately 25% of the patients in the initial course of the disease, however, airway symptoms eventually occur in 50% of all patients with RP [13]. The disorder causes airway obstruction by two mechanisms. The first is a stricture due to inflammatory swelling or scar formation in the glottic and subglottic areas. The second is dynamic airway collapse during respiration due to destruction of tracheal cartilage [14]. Accordingly, radiographic and bronchoscopic examinations may provide incorrect information for determining the degree of tracheal obstruction, as dynamic airway collapse occurs during respiration [15].

A review of few series of patients with RP illustrates the frequency of airway involvement and the female predominance (**Table 1**).

The CT findings in patients with RP consisted mainly of airway wall thickening, airway stenosis, airway malacia, airway wall calcification, and air trapping. The most common CT manifestations were increased attenuation and smooth corticosteroid sickening of airway walls. Tracheal or bronchial stenosis was less common. Airway collapse and lobar air trapping were seen in half of patients examined with expiratory CT [9].

The utility of fluorodeoxyglucose positron emission tomography/computed tomography for early diagnosis and evaluation of disease activity of RP was reported by Yamashita *et al.* It seems to be a potentially powerful tool for the early diagnosis of RP, especially in patients without easily biopsied organ involvement [21]. Respiratory function test is sensitive in early detection of airway involvement in RP. Bronchoscopy and CT are useful

Table 1. Review of literature of RP cases.

Author	n	Period	Age	Female	Airway involvement	Deaths
Gao L *et al.* [16]	23	1996-2011	-	-	10	3
Hong G *et al.* [17]	12	2004-2011	48	5	12	9
Zhang JQ *et al.* [18]	13	2000-2006	50	4	12	1
Shi XH *et al.* [19]	56	2006	45	18	38	
Maimon N *et al.* [20]	3	1995-2007	44	3	3	2

in evaluation of the severity of airway involvement in patients with RP.

In this case, subglottic stenosis was found during a bronchoscopic examination and emergency tracheostomy was performed. Other chondritis and bilateral scleritis developed 3 months after tracheostomy. Chang *et al*. described the same features leading to tracheostomy but the patient's upper airway was completely collapsed [22]. In fact, tracheostomy was once the most likely surgical procedure in RP. However, this procedure is only effective in patients with upper subglottic involvement. In cases of extensive tracheobronchial involvement, tracheostomy is ineffective because the distant tracheal collapse below the tracheostomy is still unresolved [23].

There are a number of treatments available for RP. Immunosuppression including steroid medication and chemotherapy with drugs is considered the primary option. A tracheostomy or surgical interventions, such as airway stenting, are indicated for patients with aggravated tracheobronchial symptoms like our patient. Airway management such as laryngotracheal reconstruction can improve quality of life and palliate patients effectively [24].

Corticosteroids remain the major treatment. Other therapies include nonsteroidal anti-inflammatory drugs, dapsone, colchicine, azathioprine, methotrexate, cyclophosphamide, hydroxychloroquine, cyclosporine, and infliximab [25]. Corticosteroid therapy is effective in improving the symptoms and delaying the progression of relapsing polychondritis with involvement of the respiratory tract at early stage. At later stage of the disease, airway interventional therapy, such as metallic stent placement, tracheostomy or positive airway pressure support, can be used to treat airway obstruction and to improve the survival. The successful use of infliximab is reported by some authors [26].

The airway tract involvement is known to be one of the most important prognostic factors in patients with RP [27]. The possibility that airway obstruction in the initial stages of RP is due to airway inflammation and that early, aggressive immunosuppressive treatment of RP may delay or prevent irreversible cartilaginous destruction and airway collapse is discussed [28].

This case is reported with the hope of increasing awareness about the potential for early upper airway involvement in RP [23] [29]-[35].

4. Conclusion

Symptomatic airway involvement in patients with RP is common, diverse, has a female predominance, and may occur throughout the course of disease. Dynamic CT scans are useful tools for the detection and surveillance of airway involvement in this population. The treatment of airway problems encountered in patients with RP can be challenging and needs to be individualized. Therefore, accurate diagnosis and referral to a specialized center for full multidisciplinary workup, medical treatment, and possible endoscopic intervention are important.

Conflict of Interest

There are no conflicts of interest.

References

[1] Sharma, A., Gnanapandithan, K., Sharma, K. and Sharma, S. (2013) Relapsing Polychondritis: A Review. *Clinical Rheumatology*, **32**, 1575-1583. http://dx.doi.org/10.1007/s10067-013-2328-x

[2] Chopra, R., Chaudhary, N. and Kay, J. (2013) Relapsing Polychondritis. *Rheumatic Disease Clinics of North America*, **39**, 263-276. http://dx.doi.org/10.1016/j.rdc.2013.03.002

[3] Damiani, J.M. and Levine, H.L. (1979) Relapsing Polychondritis—Report of Ten Cases. *Laryngoscope*, **89**, 929-946. http://dx.doi.org/10.1288/00005537-197906000-00009

[4] Keidel, S., McColl, A. and Edmonds, S. (2011) Sweet's Syndrome after Adalimumab Therapy for Refractory Relapsing Polychondritis. *BMJ Case Rep*, 2011.

[5] Fujiki, F., Tsuboi, Y., Hashimoto, K., Nakajima, M. and Yamada, T. (2004) Nonherpetic Limbic Encephalitis Associated with Relapsing Polychondritis. *Journal of Neurology, Neurosurgery & Psychiatry*, **75**, 1646-1647. http://dx.doi.org/10.1136/jnnp.2003.035170

[6] Yi, H. and Dong, M. (2014) Clinical Analysis of 26 Patients with Relapsing Polychondritis. *Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*, **28**, 446-449.

[7] Lee, K.S., Ernst, A., Trentham, D.E., Lunn, W., Feller-Kopman, D.J. and Boiselle, P.M. (2006) Prevalence of Functional Airway Abnormalities in Relapsing Polychondritis. *Radiology*, **240**, 565-573.

http://dx.doi.org/10.1148/radiol.2401050562

[8] McAdam, L.P., O'Hanlan, M.A., Bluestone, R. and Pearson, C.M. (1976) Relapsing Polychondritis: Prospective Study of 23 Patients and a Review of the Literature. *Medicine* (*Baltimore*), **55**, 193-215. http://dx.doi.org/10.1097/00005792-197605000-00001

[9] Behar, J.V., Choi, Y.W., Hartman, T.A., Allen, N.B. and McAdams, H.P. (2002) Relapsing Polychondritis Affecting the Lower Respiratory Tract. *American Journal of Roentgenology*, **178**, 173-177. http://dx.doi.org/10.2214/ajr.178.1.1780173

[10] Eng, J. and Sabanathan, S. (1991) Airway Complications in Relapsing Polychondritis. *The Annals of Thoracic Surgery*, **51**, 686-692. http://dx.doi.org/10.1016/0003-4975(91)90348-T

[11] Purcelli, F.M., Nahum, A. and Monell, C. (1962) Relapsing Polychondritis with Tracheal Collapse. *Annals of Otology, Rhinology & Laryngology*, **71**, 1120-1129. http://dx.doi.org/10.1177/000348946207100426

[12] Michet Jr., C.J., McKenna, C.H., Luthra, H.S. and O'Fallon, W.M. (1986) Relapsing Polychondritis. Survival and Predictive Role of Early Disease Manifestations. *Annals of Internal Medicine*, **104**, 74-78. http://dx.doi.org/10.7326/0003-4819-104-1-74

[13] Hayward, A.W. and Al-Shaikh, B. (1988) Relapsing Polychondritis and the Anaesthetist. *Anaesthesia*, **43**, 573-577. http://dx.doi.org/10.1111/j.1365-2044.1988.tb06692.x

[14] Krell, W.S., Staats, B.A. and Hyatt, R.E. (1986) Pulmonary Function in Relapsing Polychondritis. *American Review of Respiratory Disease*, **133**, 1120-1123.

[15] Burgess, F.W., Whitlock, W., Davis, M.J. and Patane, P.S. (1990) Anesthetic Implications of Relapsing Polychondritis: A Case Report. *Anesthesiology*, **73**, 570-572. http://dx.doi.org/10.1097/00000542-199009000-00037

[16] Gao, L., Lou, W.H. and Cheng, X.L. (2012) Clinical Observation of Relapsing Polychondritis: A Report of 23 Patients. *Chinese Medical Journal*, **92**, 1126-1128.

[17] Hong, G. and Kim, H. (2013) Clinical Characteristics and Treatment Outcomes of Patients with Relapsing Polychondritis with Airway Involvement. *Clinical Rheumatology*, **32**, 1329-1335. http://dx.doi.org/10.1007/s10067-013-2279-2

[18] Lin, Z.Q., Xu, J.R., Chen, J.J., Hua, X.L., Zhang, K.B. and Guan, Y.J. (2010) Pulmonary CT Findings in Relapsing Polychondritis. *Acta Radiologica*, **51**, 522-526. http://dx.doi.org/10.3109/02841851003682036

[19] Shi, X.H., Tong, S.Q., Su, J.M., Huang, H., Zhang, F.C. and Tang, F.L. (2006) Characteristics of Airway Involvement in Relapsing Polychondritis. *Chinese Medical Journal*, **86**, 1048-1051.

[20] Maimon, N., Lee, P., Paul, N., Hwang, D., Marras, T.K., Keshavjee, S., *et al*. (2010) Tracheobronchial Involvement as a Sole Manifestation of Relapsing Polychondritis. *Journal of Bronchology & Interventional Pulmonology*, **17**, 5-10. http://dx.doi.org/10.1097/LBR.0b013e3181ca27bf

[21] Yamashita, H., Takahashi, H., Kubota, K., Ueda, Y., Ozaki, T., Yorifuji, H., *et al*. (2014) Utility of Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography for Early Diagnosis and Evaluation of Disease Activity of Relapsing Polychondritis: A Case Series and Literature Review. *Rheumatology*, **53**, 1482-1490. http://dx.doi.org/10.1093/rheumatology/keu147

[22] Chang, S.J., Lu, C.C., Chung, Y.M., Lee, S.S., Chou, C.T. and Huang, D.F. (2005) Laryngotracheal Involvement as the Initial Manifestation of Relapsing Polychondritis. *Journal of the Chinese Medical Association*, **68**, 279-282. http://dx.doi.org/10.1016/S1726-4901(09)70151-0

[23] Tso, A.S., Chung, H.S., Wu, C.Y., Li, J.Y., Hong, C.L., Yang, M.W., *et al*. (2001) Anesthetic Management of a Patient with Relapsing Polychondritis—A Case Report. *Acta Anaesthesiologica Sinica*, **39**, 189-194.

[24] Karaman, E., Duman, C., Cansz, H., Ylmaz, M. and Ibrahimov, M. (2010) Laryngotracheal Reconstruction at Relapsing Polychondritis. *Journal of Craniofacial Surgery*, **21**, 211-212. http://dx.doi.org/10.1097/SCS.0b013e3181c50f7d

[25] Rapini, R.P. and Warner, N.B. (2006) Relapsing Polychondritis. *Clinics in Dermatology*, **24**, 482-485. http://dx.doi.org/10.1016/j.clindermatol.2006.07.018

[26] Cazabon, S., Over, K. and Butcher, J. (2005) The Successful Use of Infliximab in Resistant Relapsing Polychondritis and Associated Scleritis. *Eye*, **19**, 222-224. http://dx.doi.org/10.1038/sj.eye.6701457

[27] Xie, C., Shah, N., Shah, P.L. and Sandhu, G. (2013) Laryngotracheal Reconstruction for Relapsing Polychondritis: Case Report and Review of the Literature. *The Journal of Laryngology & Otology*, **127**, 932-935. http://dx.doi.org/10.1017/s0022215113001746

[28] Segel, M.J., Godfrey, S. and Berkman, N. (2004) Relapsing Polychondritis: Reversible Airway Obstruction Is Not Always Asthma. *Mayo Clinic Proceedings*, **79**, 407-409. http://dx.doi.org/10.4065/79.3.407

[29] Eksombatchai, D., Boonsarngsuk, V., Amornputtisathaporn, N., Suwatanapongched, T. and Kurimoto, N. (2013) Tracheobronchial Involvement in Relapsing Polychondritis Diagnosed on Endobronchial Ultrasound. *Internal Medicine*, **52**, 801-805. http://dx.doi.org/10.2169/internalmedicine.52.9416

[30] Hellmich, B., Hering, S., Duchna, H.W., Schultze-Werninghaus, G., Freitag, L., Schatz, H., *et al*. (2003) Airway Manifestations of Relapsing Polychondritis: Treatment with Cyclophosphamide and Placement of Bronchial Stents. *Zeitschrift für Rheumatologie*, **62**, 73-79. http://dx.doi.org/10.1007/s00393-003-0419-9

[31] Tsunezuka, Y., Sato, H. and Shimizu, H. (2000) Tracheobronchial Involvement in Relapsing Polychondritis. *Respiration*, **67**, 320-322. http://dx.doi.org/10.1159/000029518

[32] Sakata, A., Nakahara, H. and Murofushi, T. (2006) A Case of Severe Dyspnea Caused by Relapsing Polychondritis. *Auris Nasus Larynx*, **33**, 215-218. http://dx.doi.org/10.1016/j.anl.2005.11.008

[33] Zhang, J.Q., Li, Q., Bai, C., Huang, Y., Dong, Y.C., Yao, X.P., *et al*. (2007) Clinical Features and Treatment of Relapsing Polychondritis with Involvement of the Respiratory Tract-Report of Thirteen Cases. *Chinese Journal of Tuberculosis and Respiratory Diseases*, **30**, 173-177.

[34] Kim, I.K., Kim, M.S., Choi, Y.S. and Shin, Y.S. (2012) Anesthetic Experience of a Patient with Relapsing Polychondritis—A Case Report. *Korean Journal of Anesthesiology*, **63**, 465-468. http://dx.doi.org/10.4097/kjae.2012.63.5.465

[35] Azuma, N. (2012) Airway Involvement in Relapsing Polychondritis. *Nihon Rinsho Meneki Gakkai Kaishi*, **35**, 157-167. http://dx.doi.org/10.2177/jsci.35.157

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Hemoglobin Level Stability after a Switch from Darbepoetin Alfa to Epoetin Beta Pegol for the Treatment of Renal Anemia in Hemodialysis Patients

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Abstract

Background: New erythropoiesis-stimulating agents (ESAs) with a longer half-life have been developed for the treatment of anemia as a complication of patients with end-stage renal disease. Objectives: The objective of the present study was to assess the hemoglobin (Hb) stability of a Japanese cohort of hemodialysis (HD) patients who were simultaneously switched from darbepoetin alfa (DA) to epoetin beta pegol (CERA). Methods: This was an observational, prospective study of HD patients 20 years of age or more who were switched from intravenous (IV) DA to IV CERA and continued on HD for at least 3 months. The dose was adjusted to maintain the Hb level to within 1.0 g/dl of the baseline value. Results: A total of 68 HD patients (75.0% male, median age 63.0 years) were enrolled. The patients' mean Hb levels were 10.8 ± (0.6) g/dl at Month 0, 10.9 ± 0.7 at Month 1, 10.8 ± 0.7 at Month 2, and 10.9 ± 0.8 at Month 3, and the differences from the level at Month 0 were not significant. After the switch, the ESA dose decreased significantly ($P < 0.0001$) from an annual mean DA dose of 549.0 ± 246.6 IU/kg/month to a mean CERA dose at Month of 3 491.0 ± 291.7 IU/kg/month. The ESA resistance index (ERI) decreased from 51.7 ± 24.4 IU/kg/month/g/dl on DA at Month 0 to 46.4 ± 29.3 on CERA at 3 Month 3 ($P < 0.0001$). Conclusion: Switching from DA to CERA was associated with approximate 89% reduction of the required dose in Japanese HD patients being treated with an ESA and showed a favorable impact on the treatment of renal anemia, including the need for less frequent injections and a reduction of the ESA dose.

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Keywords

Darbepoetin Alfa, Epoetin Beta Pegol, Hemoglobin, ESA Resistance, Hemodialysis

1. Introduction

Since renal anemia is a complication of advanced chronic kidney disease (CKD), administration of erythropoiesis-stimulating agents (ESAs) has led to a great improvement in clinical management [1], because ESA treatment significantly reduces the need for transfusions, hospital admissions, and overall mortality [2]-[4]. The first ESA available for clinical use namely epoetin alfa and beta, had a short half-life and needed to be administered twice or thrice a week [5]. Although ESAs are basically administered thrice a week to hemodialysis (HD) patients, anemia is the complication that has the greatest impact on perceived quality of life in HD patients.

Dalbepoetin alfa (DA) has a much longer half-life and longer erythropoietic effect than epoetin alfa [6] [7]. The initially recommended conversion rate was 200 IU of epoetin alfa per 1 μg of DA [8]. Nevertheless, administration of DA at 2-week intervals is necessary in patients whose anemia is not adequately improved by DA therapy at a 4-week dosing interval. Continuous erythropoietin receptor activator (CERA) is a newer ESA that has a long half-life and requires less frequent dosing, *i.e.*, once every two weeks or even once monthly [9]. CERA has a longer half-life, approximately 134 hours, when administered intravenously to HD patients [10].

However, it is uncertain if switching HD patients from DA to CERA stabilizes their target Hb level. The aim of the present study was to assess the effects of switching from DA to CERA on the Hb level of a cohort of Japanese HD patients with renal anemia.

2. Materials

2.1. Subjects and Protocol

This was a prospective cohort study conducted at a single center in Japan. The subjects were recruited from among patients who had been routinely treated through an arteriovenous fistula in the dialysis unit of the Shinjuku Ishikawa Clinic for at least 6 months. The Institutional Review Board of the Shinjuku Ishikawa Clinic approved all study protocols, and they were performed in accordance with the Declaration of Helsinki guidelines regarding ethical principles for medical research involving human subjects. Informed consent was obtained from all of the subjects [11].

HD patients who had malignancy, active inflammation, liver cirrhosis, gastrointestinal bleeding, or severe illness were excluded from participation. The patients who enrolled as subjects (n = 68) were undergoing stable regular HD with a bicarbonate dialysate. Their underlying renal diseases were chronic glomerulonephritis (38.2%), diabetic nephropathy (25.0%), hypertensive nephrosclerosis (5.9%), and others (30.9%). All patients were on thrice-weekly HD, and no further patient selection was performed. The main inclusion criteria were 20 years of age or more, a switch from DA to CERA (index date) after being on HD for at least 6 months, and having remained on HD for at least 3 months after the switch.

The date of the switch from DA to CERA was considered the index date (Month 0). The baseline conversion rate was left to the discretion of each nephrologist. Both ESAs were intravenously injected with pre-filled syringes. The DA dose frequency was once weekly, and CERA was administered twice monthly. Hb was measured monthly, and the ESA dose was adjusted to maintain the Hb level to within 1.0 g/dl of the Month-0 value. The target Hb was 10 - 12 g/dl, and iron supplementation was started when the serum ferritin level was <100 ng/mL or transferrin saturation (TSAT) was <20%, as recommended by the Japanese Society for Dialysis Therapy (JSDT) [12]. The ESA resistance index (ERI) was calculated by dividing the ESA dose by the Hb level [13] [14].

The prospective follow-up period was 3 months. The DA and CERA doses were converted to IU/kg/month by using the 200:1 conversion rattio of epoetin alfa. We collected data on Hb, serum ferritin, TSAT, ESA dose, and ERI. Only adverse reactions leading to discontinuation of CERA were recorded.

2.2. Statistical Analysis

Normally distributed, unpaired continuous values were expressed as means ± SD and compared by performing

an analysis of variance. Nonparametric values were expressed as median values and compared by performing the Kruskal-Wallis test. Categorical values were expressed as percentages and compared by performing Fisher's exact test. Changes from Month 0 values at subsequent visits were evaluated by using the paired *t*-test and Wilcoxon signed-rank test. Changes in continuous variables over time were evaluated by repeated-measures analysis of variance. The statistical analysis was performed by using SAS software (v 9.2; SAS Institute, Cary, NC, USA).

3. Results

3.1. Patients

There were 126 HD patients at the index date, and 68 of them were included in this study. Subjects who did not complete the study because of insufficient data or transfer to another dialysis institute were excluded. Patient characteristics are summarized in **Table 1**. The median age of the 68 subjects was 63 years (53 - 68 years), and their median dialysis vintage was 144 months (85 - 227 months). Their prevalence of diabetes was 25.0%. The study population included 17 women and 51 men.

3.2. Hb Levels over Time

No significant changes in mean monthly Hb values were observed during the follow-up period (10.8 ± 0.6 at Month 0, 10.9 ± 0.7 at Month 1, 10.8 ± 0.7 at Month 2, 10.9 ± 0.8 at Month 3; **Table 2**). Only a slight increase in mean Hb level was observed at 5 weeks after the switch and the Hb level rapidly returned to its baseline level after minor dose adjustments at 7 weeks after the start of CERA administration (**Figure 1**). There were no significant changes over time in the number of bleedings or transfusions (data not shown). A bleeding episode was experienced by <1% and none of the patients required a blood transfusion after the switch. CERA was not discontinued because adverse reactions in any of the patients during the follow-up period.

We compared the Hb levels and ESA doses of a diabetic group (n = 16) and nondiabetic group (n = 45) at the time of the switch and at the end of the follow-up period. There were no significant changes in the Hb level in the diabetic group (Month 0: 10.5 ± 0.6 vs. Month 3: 10.6 ± 0.7 g/dl) or in the nondiabetic group (Month 0: 10.8 ± 0.7 vs. Month 3: 10.8 ± 0.8 g/dl). Nor were there any significant changes in the ESA doses in the diabetic group (Month 0: 524.9 ± 245.8 vs. Month 3: 498.0 ± 258.6 IU/kg/month) nor nondiabetic group (Month 0: 557.6 ± 249.0 vs. Month 3:507.7 ± 303.5 IU/kg/month), suggesting that there was no difference in the effect of CERA in the two groups.

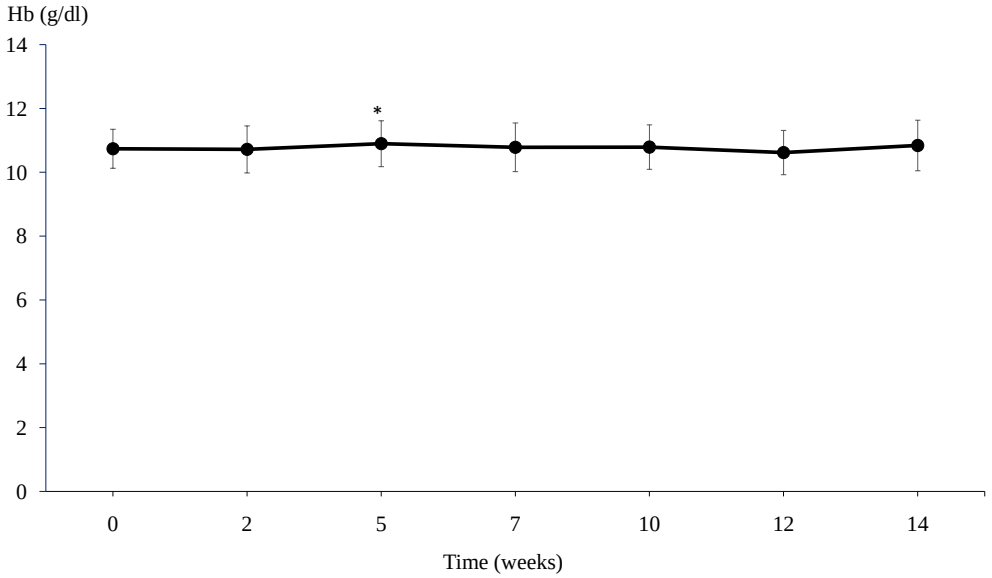

Figure 1. Changes in hemoglobin levels after switching from dalbepoetin alfa to epoetin beta pegol. *$P < 0.05$ vs. baseline.

Table 1. Baseline characteristics of the study population.

	(n = 68)
Age, years	63 (53 - 68)
Male gender, %	51 (75.0)
Dialysis vintage, months	144 (85 - 227)
CKD etiology, %	
Diabetes mellitus	25.0
Glomerulonephritis	38.2
Nephrosclerosis	5.9
Others	30.9
Smoking, %	27.9
History of cardiovascular disease[1], %	42.6
Body mass index, kg/m^2	21.8 (19.2 - 24.2)
Mean blood pressure, mmHg	99.0 (90.3 - 107.9)
Pulse pressure, mmHg	68 (59 - 80)
Single pool Kt/V	1.5 (1.3 - 1.6)
Hemoglobin, g/dl	10.7 (10.5 - 11.0)
Albumin, g/dl	3.8 (3.6 - 4.0)
C-reactive protein, mg/dl	0.12 (0.05 - 0.23)
Total cholesterol, mg/dl	148 (130 - 169)
HDL-cholesterol, mg/dl	44 (35 - 53)
Non-HDL-cholesterol, mg/dl	103 (82 - 123)
Triglyceride, mg/dl	93 (63 - 121)
Calcium, mg/dl	9.1 (8.9 - 9.4)
Phosphorus, mg/dl	5.4 (4.6 - 6.4)
Intact parathyroid hormone, pg/ml	123 (86 - 181)
NT-proBNP, pg/ml	1990 (4634 - 10,255)
Antihypertensive agents	
RAS inhibitors, %	69.1
CCBs, %	60.3
Beta-blockers, %	48.5
Statin, %	25.0
EPA, %	11.8
$CaCO_3$, %	85.3
Phosphate binders, %	70.6

Data are expressed as median (range) or percentage. [1]History of heart disease has been defined as congestive heart failure determined by echocardiography within the 3 previous months, myocardial infarction, cerebrovascular disease and peripheral artery disease. Abbreviations: CKD, chronic kidney disease; HDL, high-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RAS, renin-angiotensin system; CCB, calcium channel blocker; EPA, eicosapentaenoic acid.

3.3. ESA doses and ERI over time

After the switch from DA to CERA, the ESA dose decreased abruptly and significantly ($P < 0.001$), and remained unchanged thereafter (**Figure 2**). At the time of the switch, the mean ESA dose was 549.0 ± 246.6 IU/kg/month, and a significant decrease from the dose at the time of the switch was observed at Month 1 (517.6 ± 259.6 IU/kg/month), Month 2 (510.0 ± 278.2 IU/kg/month), and Month 3 (491.0.0 ± 291.7 IU/kg/month) ($P < 0.05$) (**Table 2**).

The reduction in ESA dose was significantly greater in the low-dose group at baseline (<420 IU/kg/month) than in the high dose group (≥420 IU/kg/month) (**Table 3**).

The ERI decreased from an annual mean of 51.7 ± 24.4 IU/kg/month/g/dl on DA to 48.6 ± 25.8 IU/kg/month/g/dl at Month 1, 48.3 ± 27.4 IU/kg/month/g/dl at Month 2, and 46.4 ± 29.3 IU/kg/month/g/dl at Month 3 ($P < 0.05$) (**Table 2**, **Figure 3**).

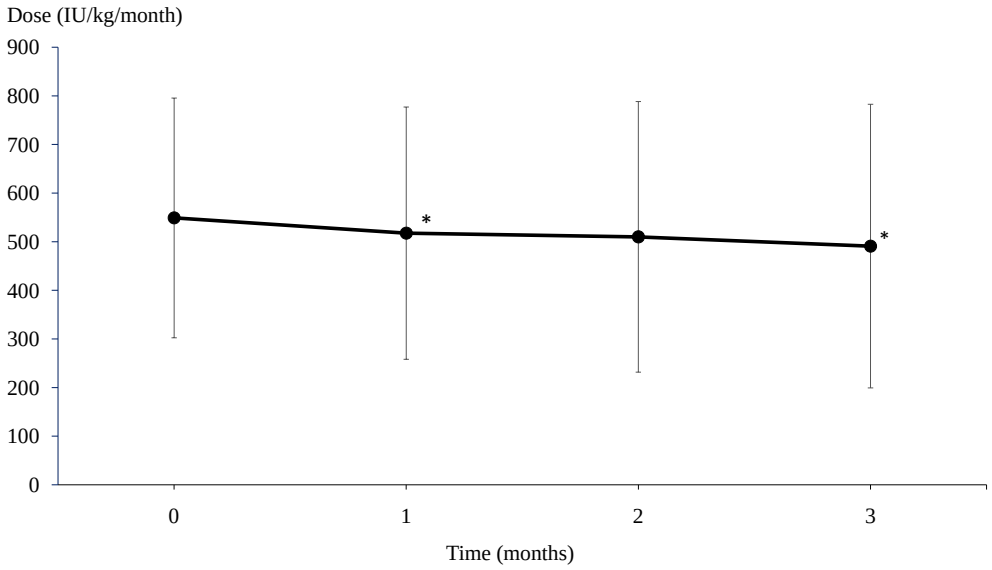

Figure 2. Changes in erythropoiesis-stimulating agent (ESA) dose after switching from dalbepoetin alfa to epoetin beta pegol. $^{*}P < 0.05$ vs. baseline.

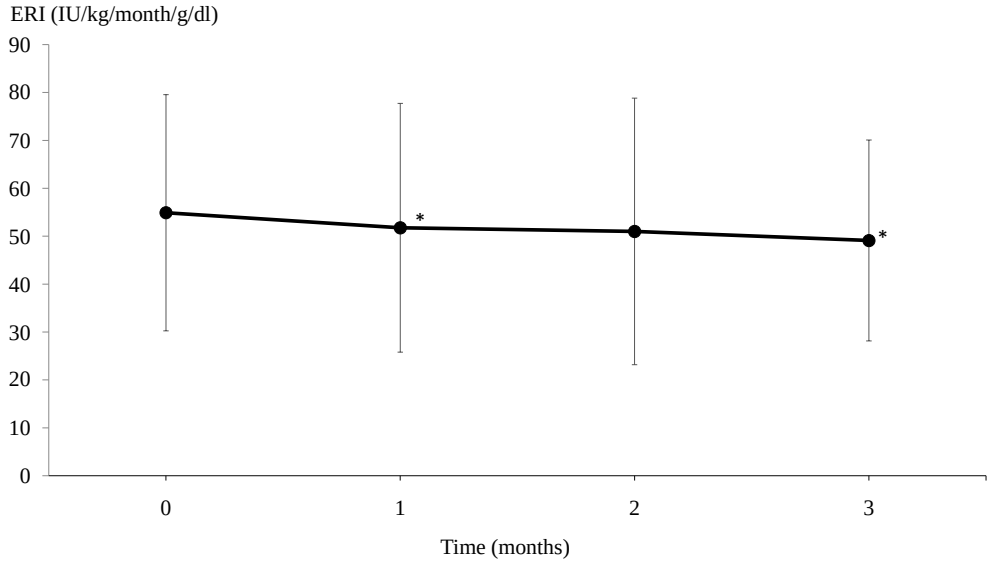

Figure 3. Changes in erythropoiesis-stimulating agent resistance index (ERI) after switching from dalbepoetin alfa to epoetin beta pegol. $^{*}P < 0.05$ vs. baseline.

Table 2. Changes in hematological parameters after switching of ESA.

	0 month	1st month	2nd month	3rd month
Hemoglobin (g/dl)	10.8 ± 0.6	10.9 ± 0.7*	10.8 ± 0.7	10.9 ± 0.8
TSAT (%)	17.0 ± 6.5	25.0 ± 7.8**	24.5 ± 8.7**	18.6 ± 7.8
Ferritin (ng/ml)	52.7 ± 30.0	68.3 ± 50.0*	61.2 ± 39.5	57.6 ± 34.6
ESA dose (IU/kg/month)	549.0 ± 246.6	517.6 ± 259.6*	510.0 ± 278.2	491.0 ± 291.7*
ERI (IU/kg/month/g/dl)	51.7 ± 24.4	48.6 ± 25.8*	48.3 ± 27.4	46.4 ± 29.3*
TSAT (month average)	17.9 ± 6.3	22.1 ± 7.2**	22.0 ± 7.2**	18.6 ± 7.8
Ferritin (month average)	50.9 ± 22.7	59.4 ± 40.0	57.7 ± 34.0	57.6 ± 34.6

*$P < 0.05$, **$P < 0.001$ vs. 0 month. Abbreviations: TSAT, transferrin saturation time; ESA, erythropoiesis-stimulating agent; ERI, erythropoiesis-stimulating agent resistance index.

Table 3. Changes in erythropoiesis-stimulating agent (ESA) dose and ESA resistance index (ERI).

	Dose of epoetin at conversion		
	Low (<420 IU/kg/month) (n = 30)	High (≥420 IU/kg/month) (n = 31)	Total (n = 61)
ESA dose (IU/kg/month), mean (SD)			
Darbepoetin alfa	310.3 (79.0)	641.8 (178.5)	478.8 (216.5)
Epoetin beta pegol			
At the time of switch	390.7 (111.9)	702.3 (245.3)	549.0 (246.6)
First month	380.0 (142.9)	650.8 (278.8)	517.6 (259.6)
Second month	366.1 (195.5)	649.3 (277.7)	510.0 (278.2)
Third month	338.7 (244.4)	638.4 (258.3)	491.0 (291.7)
ESA dose reduction (%), mean (SD)			
At the time of switch	100.0	100.0	100.0
First month	99.5 (40.6)	90.7 (16.0)	95.0 (30.7)
Second month	95.5 (46.9)	90.8 (26.2)	93.1 (37.6)
Third month	86.3 (58.5)	90.7 (28.3)	88.5 (45.4)
ERI (IU/kg/month/g/dl), mean (SD)			
Darbepoetin alfa	29.2 (7.6)	62.3 (19.1)	46.0 (22.1)
Epoetin beta pegol			
At the time of a change	36.5 (11.0)	66.4 (25.0)	51.7 (24.4)
First month	35.6 (14.7)	61.2 (28.1)	48.6 (25.8)
Second month	34.5 (19.7)	61.7 (27.4)	48.3 (27.4)
Third month	32.3 (25.7)	60.0 (26.3)	46.4 (29.3)
ERI reduction (%), mean (SD)			
At the time of a change	100.0	100.0	100.0
First month	99.7 (44.9)	89.9 (16.4)	94.7 (33.7)
Second month	96.2 (50.1)	91.1 (27.3)	93.6 (39.9)
Third month	87.2 (61.6)	90.3 (30.9)	88.7 (48.1)

3.4. Changes in Serum Ferritin and TSAT

There were no significant changes in serum ferritin levels during the follow-up period (**Figure 4(a)**), but temporal changes in TSAT (**Figure 4(b)**) were observed during the follow-up period, suggesting that there was no change in iron metabolism after switching from DA to CERA.

4. Discussion

The present study investigated changes in the Hb levels of Japanese HD patients after switching from DA to CERA. The mean ESA dose gradually decreased after the switch, and the reduction was sustained during the subsequent 3 months without any noticeable changes in Hb levels. The mean ERI values had also significantly decreased at the end of the follow-up period. These findings support the results of a previous study by Hirai *et al.* [15].

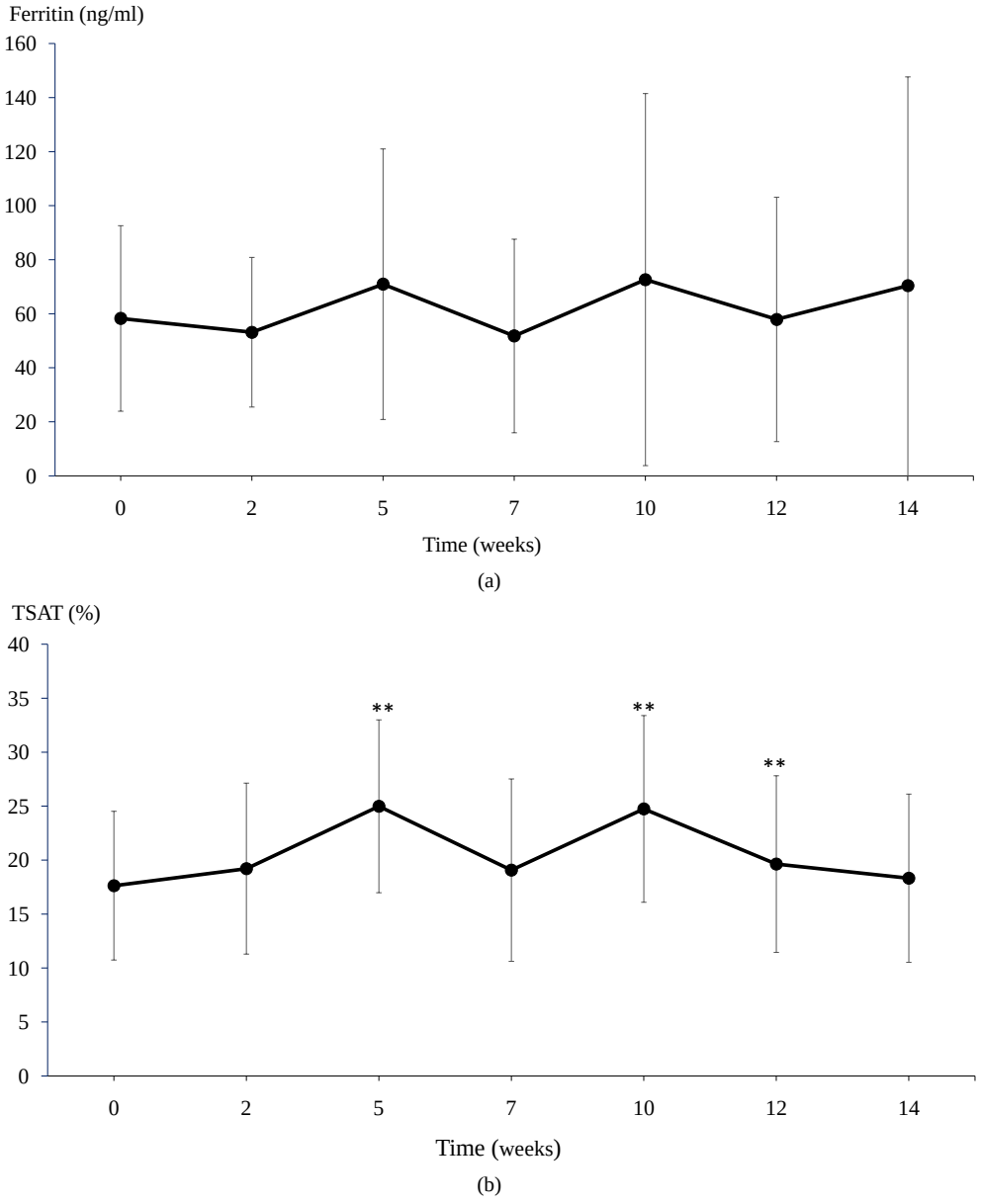

Figure 4. Changes in serum ferritin levels (a) and transferrin saturation (TSAT, (b)) after switching from dalbepoetin alfa to epoetin beta pegol. $^{*}P < 0.05$ vs. baseline.

We used the manufacturer's recommended starting dose of CERA when we switched the patients from DA to CERA. There are no guidelines for switching from DA to CERA in Japan [12]. The mean Hb levels were generally maintained within the target range (10.0 - 12.0 g/dl) during the follow-up period after the switch. Hb variability [16] and ESA overdosing are important issues for the treatment of renal anemia. In our study, the Hb level had increased slightly at 5 weeks after the switch, but the reduction in CERA dose necessary to maintain the target Hb levels appeared. A lower starting dose of CERA may reduce Hb variability after switching from DA.

The conversion ratio based on the actual doses of DA and CERA was estimated to be 1.0:1.0 at the time of the switch. However, the CERA dose gradually decreased from 549.0 ± 246.6 IU/kg/month at the baseline to 491.0.0 ± 291.7 IU/kg/month (89%) during the follow-up period. This finding is evidence that CERA should be started at approximately 80% - 90% of the DA dose to maintain equivalent Hb levels when the switching ESA from DA to CERA, suggesting that the CERA dose is reducible during the maintenance phase of the CERA therapy [17]. However, determination of the optimal dose of CERA will require further investigation.

Morikami *et al.* have recently reported finding that biweekly administration of CERA showed a significant decrease in serum ferritin levels in association with temporal changes in TSAT in Japanese HD patients [18]. The results of the present study showed that switching from DA to CERA did not alter the subjects' serum ferritin levels with a slight fluctuation in TSAT levels during the follow-up period. The serum ferritin levels of our patients were maintained at low levels. When switching from DA to CERA, iron metabolism may not be changed with regard to erythropoiesis, suggesting that biweekly CERA administration is advantageous in regard to iron absorption and utilization, and would be preferable in the management of renal anemia in HD patients.

There were several limitations in this study that should be considered. First, this study was based on a relatively small sample of HD patients from a single center, thereby limiting the possibility of generalizing our findings. Second, we excluded around 50% of all of the prevalent HD patients from the analysis and there was a possible bias due to the inclusion of survivors.

5. Conclusion

The results of this study demonstrated that switching from DA to CERA was associated with an 89% reduction of the required ESA dose in Japanese HD patients being treated with an ESA for renal anemia and they showed that the switch had a favorable impact on treatment of renal anemia, including a need for less frequent injections and reduction of the ESA dose.

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Disclosure

The authors have no conflicts of interest to declare.

References

[1] Eschbach, J.W. and Adamson, J.W. (1988) Recombinant Human Erythropoietin: Implications for Nephrology. *American Journal of Kidney Diseases*, **11**, 203-209. http://dx.doi.org/10.1016/S0272-6386(88)80150-1

[2] Macdougall, I.C. (2002) Optimizing the Use of Erythropoietic Agents—Pharmacokinetic and Pharmacodynamic Considerations. *Nephrology Dialysis Transplantation*, **17**, 66-70. http://dx.doi.org/10.1093/ndt/17.suppl_5.66

[3] Locatelli, F., Aljama, P., Barany, P., Canaud, B., Carrera, F., Eckardt, K.U., *et al.* (2004) Revised European Best Practice Guidelines for the Management of Anaemia in Patients with Chronic Renal Failure. *Nephrology Dialysis Transplantation*, **19**, ii1-ii47.

[4] Fishbane, S. (2010) The Role of Erythropoiesis-Stimulating Agents in the Treatment of Anemia. *American Journal of Managed Care*, **16**, S67-S73.

[5] Halstenson, C.E., Macres, M., Katz, S.A., Schnieders, J.R., Watanabe, M., Sobota, J.T. and Abraham, P.A. (1991) Comparative Pharmacokinetics and Pharmacodynamics of Epoetin Alfa and Beta. *Clinical Pharmacology and Therapeutics*, **50**, 702-712. http://dx.doi.org/10.1038/clpt.1991.210

[6] Macdougall, I.C. (2002) Dalbepoetin Alfa: A New Therapeutic Agent for Renal Anemia. *Kidney International Suppl.*, **61**, S55-S61. http://dx.doi.org/10.1046/j.1523-1755.61.s80.11.x

[7] Hudson, J.Q. and Sameri, R.M. (2002) Dalbepoetin Alfa, a New Therapy for the Management of Anemia of Chronic Kidney Disease. *Pharmacotherapy*, **22**, 141S-149S. http://dx.doi.org/10.1592/phco.22.14.141S.33397

[8] Aljama, P., Bommer, J., Canaud, B., Carrera, F., Eckardt, K.U., Horl, W.H., *et al.* (2001) Practical Guidelines for the Use of HESP in Treating Renal Anaemia. *Nephrology Dialysis Transplantation*, **16**, 22-28. http://dx.doi.org/10.1093/ndt/16.suppl_3.22

[9] Macdougall, I.C., Gray, S.J., Elston, O., Breen, C., Jenkins, B., Browne, J. and Egrie, J. (1999) Pharmacokinetics of Novel Erythropoiesis Stimulating Protein Compared with Epoetin Alfa in Dialysis Patients. *Journal of American Society of Nephrology*, **10**, 2392-2395.

[10] Locatelli, F. and Reigner, B. (2007) C.E.R.A.: Pharmacodynamics, Pharmacokinetics and Efficacy in Patients with Chronic Kidney Disease. *Expert opinion on Investigational Drugs*, **16**, 1649-1661. http://dx.doi.org/10.1517/13543784.16.10.1649

[11] Takahashi, S., Suzuki, K., Kojima, F., Tanaka, Y. and Nitta, K. (2015) Geriatric Nutritional Risk Index as a Simple Predictor of Mortality in Maintenance Hemodialysis Patients: A Single Center Study. *International Journal of Clinical Medicine*, **6**, 354-362. http://dx.doi.org/10.4236/ijcm.2015.65046

[12] Tsubakihara, Y., Nishi, S., Akiba, T., Hirakata, H., Iseki, K., Kubota, M., *et al.* (2010) 2008 Japanese Society for Dialysis Therapy: Guidelines for Renal Anemia in Chronic Kidney Disease. *Therapeutic Apheresis and Dialysis*, **14**, 240-275. http://dx.doi.org/10.1111/j.1744-9987.2010.00836.x

[13] Okazaki, M., Komatsu, M., Kawaguchi, H., Tsuchiya, K. and Nitta, K. (2014) Erythropoietin Resistance Index and the All-Cause Mortality of Chronic Hemodialysis Patients. *Blood Purification*, **37**, 106-112. http://dx.doi.org/10.1159/000358215

[14] Ogawa, T., Shimizu, H., Kyono, A., Sato, M., Yamashita, T., Otsuka, K. and Nitta, K. (2014) Relationship between Responsiveness to Erythropoietin-Stimulating Agent and Long-Term Outcomes in Chronic Hemodialysis Patients: A Single Center Cohort Study. *International Urology and Nephrology*, **46**, 151-159. http://dx.doi.org/10.1007/s11255-013-0494-z

[15] Hirai, T., Nishizawa, Y., Nakazono, H., Asai, M., Yamashita, H., Sasaki, A., *et al.* (2013) Hemoglobin Maintenance and Dosing Strategies Using Intravenous Continuous Erythropoietin Receptor Activator in Japanese Hemodialysis Patients. *Therapeutic Apheresis and Dialysis*, **17**, 498-503. http://dx.doi.org/10.1111/1744-9987.12013

[16] Fishbane, S. and Berns, J.S. (2007) Evidence and Implications of Haemoglobin Cycling in Anemia Management. *Nephrology Dialysis Transplantation*, **22**, 2129-2132. http://dx.doi.org/10.1093/ndt/gfm384

[17] Besarab, A., Salifu, M.O., Lunde, N.M., Bansal, V., Fishbane, S., Dougherty, F.C., *et al.* (2007) Efficacy and Tolerability of Continuous Erythropoietin Receptor Activator: A 19-Week, Phase II, Multicenter, Randomized, Open-Label, Dose-Finding Study with a 12-Month Extension Phase in Patients with Chronic Renal Disease. *Clinical Therapy*, **29**, 626-639. http://dx.doi.org/10.1016/j.clinthera.2007.04.014

[18] Morikami, Y., Fujimori, A., Okada, S., Kumei, M., Mizobuchi, N. and Sakai, M. (2014) Comparison of 2-Week versus 4-Week Dosing Intervals of Epoetin Beta Pegol on Erythropoiesis and Iron Metabolism in Hemodialysis Patients. *Therapeutic Apheresis and Dialysis*, **18**, 414-420. http://dx.doi.org/10.1111/1744-9987.12164

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Recurrence of Cortical Venous Haemangioma in the Tibia: Interest of Sclerotherapy

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Abstract

We report on a rare case of an intra-osseous haemangioma involving the medial portion of tibia in a 29-year-old man. The aim is to emphasize on diagnosis difficulties, possible recurrence and interest of sclerotherapy. In radiographs, the lesion was a well-defined osteolytic lesion having 3 mm of diameter with perimeter's sclerosis. The computed tomography images demonstrated an intra-medullar gap's image of the tibia with cortical participation. An osteoid osteoma was evocated but eliminated by the histhological examination. The osseous scintigraphy didn't find any anomaly. The outcome was marked by the recurrence of pain. T1-weighted Magnetic resonance imaging showed a hypointense lesion, while T2-weighted images revealed hyperintense areas, with internal, hypointense septa. Gadolinium-enhanced T1-weighted images showed lattice-like enhancement of the lesion. These images signed a recurrence of the intra-osseous venous haemangioma. The sclerotherapy improved the pain in three days and prevented another recurrence.

Keywords

Intra-Osseous Haemangioma, Computed Tomography, Magnetic Resonance Imaging, Tibia, Sclerotherapy

1. Introduction

The majority of haemangiomas that involve bone are discovered incidentally in asymptomatic patients [1]-[4].

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Men are affected twice as often as women, and lesions are usually discovered in the 4th-5th decades of life. Soft-tissue components may also be associated with these lesions. Osseous haemangioma is particularly common in the spine and calvaria and less frequently affects long bones such as the tibia, femur, and humerus [1]-[4].

2. Case Report

A 29-year-old patient, with no particular history, complained of pain in the right leg 20 days ago with a nocturnal recrudescence, with neither fever nor trauma. The clinical examination revealed a good general condition, a painful centimetric antero-internal tumefaction of the right leg, having an osseous consistency without inflammatory signs.

Conventional radiography of the leg showed an intracortical hair image of medial cortical with peripheral sclerosis (**Figure 1**). A computed tomographic scan revealed a radiolucent intramedullary gap's image of the antero-internal cortex with longitudinal spotty internal calcification (**Figure 2(a)**, **Figure 2(b)**). The osseous scintigraphy was without hyper-fixation anomaly. A clinical diagnosis of atypical intramedullary osteoid osteoma was retained. The lesion was completely excised for histological examination. No reconstruction was necessary after the excision. A pathological diagnosis of intramedullary veinous haemangioma was retained. The outcome was marked four months after by recurrence of leg pain. The radiography and the computed tomography scan found a recurrence of the lesion. T1-weighted Magnetic resonance imaging showed a hypo intense lesion, while T2-weighted images revealed hyper intense areas, with an internal hypointense lesion. Gadolinium-enhanced T1-weighted images showed a lattice-like enhancement of the lesion (**Figure 3(a)**, **Figure 3(b)**). These images proved the recurrence of the intra-osseous venous haemangioma; Technique: A sclerotherapy guided by the computed tomography scan under local anesthesia, scannographic identification of the lesion was carried out bya transcortical pin (**Figure 4(a)**). The journey was enlarged by a strawberry (**Figure 4(b)**). A catheterization of the angioma was performed by a probe guided by the pin (**Figure 4(c)**).

Sclerotherapy under pressure with scannographic control was finally performed. This technique improved the osseous pain which disappeared within 72 hours. The 18-month follow-up was characterised by the absence of pain and the professional activities were resumed.

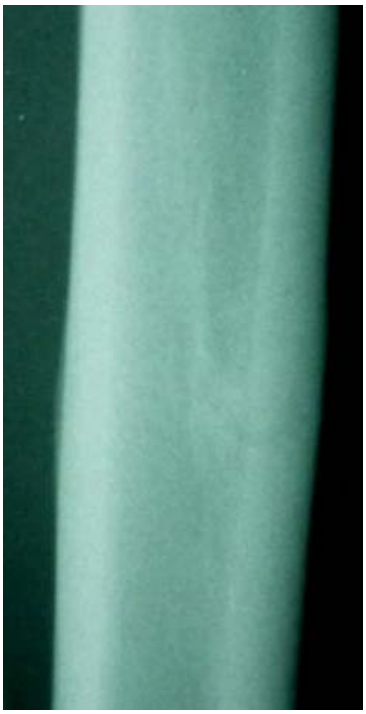
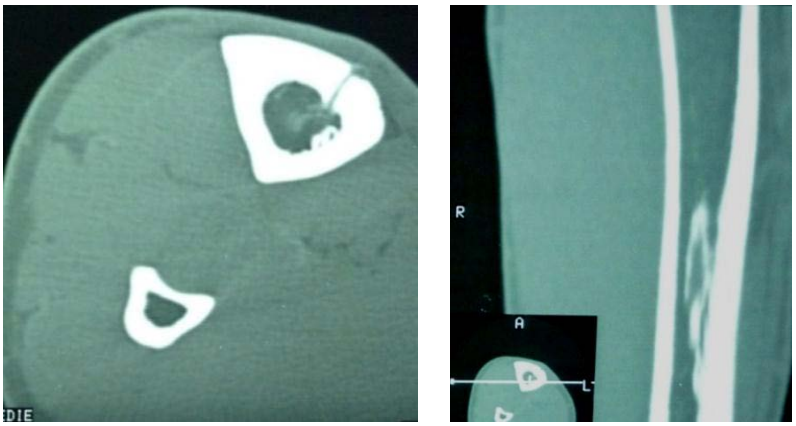
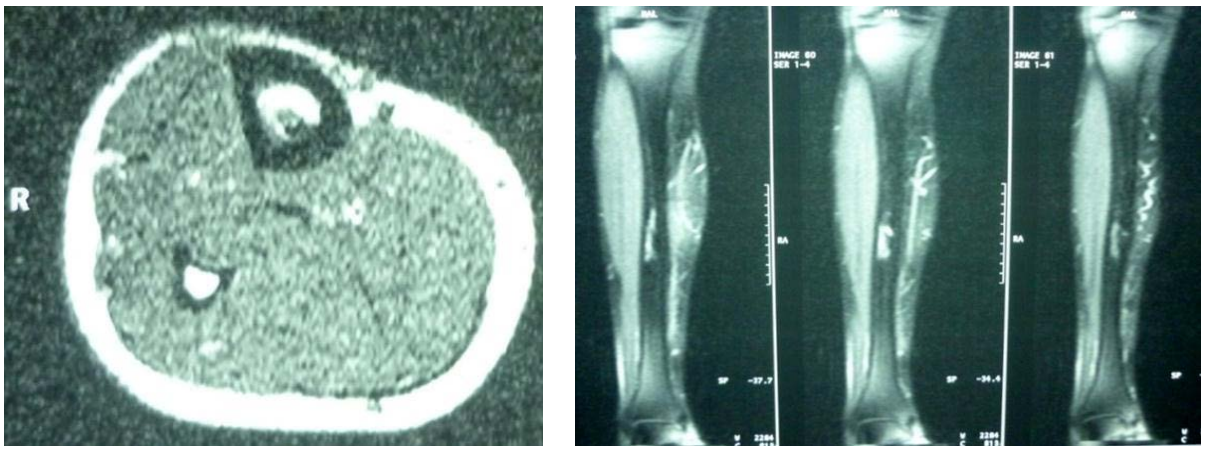


Figure 1. Intracortical medial crack lesion of leg with characteristic condensation.



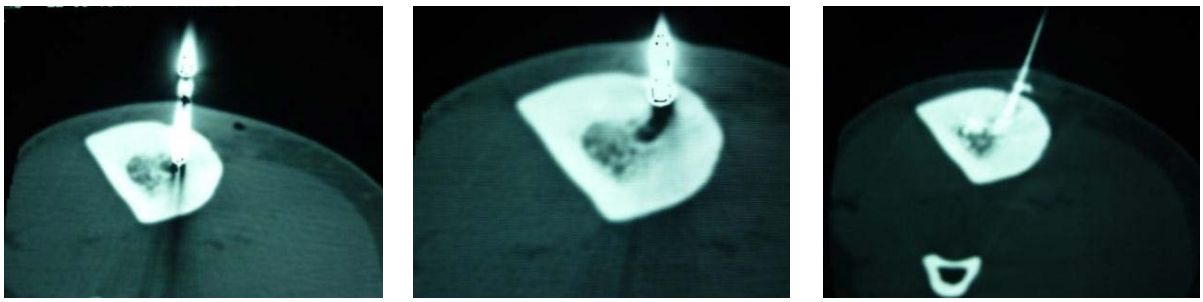
(a) (b)

Figure 2. (a) Axial computed tomographic section, one millimeter thick, showing radiolucent intramedullary gap's image; (b) Longitudinal computed tomographic section showed spotty internal calcification.



(a) (b)

Figure 3. (a) T1-weighted magnetic resonance image revealing an intramedullary area of hypo signal intensity, with internal, high intense lesion; (b) T2-weighted magnetic resonance image revealing an intramedullary segmental area of high signal intensity.



(a) (b) (c)

Figure 4. (a) Catheterization of angioma by a perforated wick guided by a primary trans-cortical pin; (b) The journey was enlarged by a strawberry; (c) Embolisation of intra-medullary angioma.

3. Discussion

A haemangioma is an abnormal proliferation of blood vessels that may occur in any vascularised tissue [1]-[4]. Controversial debate is raised as to whether these lesions are neoplasms, hamartomas, or vascular malformations.

"Haemangiomas" affecting the musculoskeletal system are more accurately termed "vascular malformations." These are present from birth and do not progress spontaneously [1].

Venous haemangiomas are composed of thick-walled vessels containing muscles and they rarely involve the musculoskeletal system. These lesions are generally found in adults and are most frequently located in the deep soft tissues such as the retro peritoneum, mesentery, and muscles of the lower extremities. They often have a slow blood flow, and phleboliths may be present [1]. Haemangioma of the bone is a rare lesion accounting for approximately 1% of all bone tumors [4]. It may involve the intramedullary canal, the cortical bone, or only the periosteum [5]. These lesions are found most frequently in the vertebrae and skull, most being asymptomatic and discovered only incidentally; they account for 25% of all skeletal haemangiomas; they occur more frequently in young women and they do not usually require any treatment [5]-[7].

In the long bones, the point of beginning is medullary in 50% of cases, periosteal in 33% of cases and cortical in 12% of cases. The lesion is in the diaphysis or the metaphyso-diaphysis in 80% of cases and it is isolated metaphysic in 10% of cases [5]. The possibility of long bone haemangioma is usually not taken into account in the initial diagnosis [8].

Osseous haemangioma which is particularly common in the spine and calvaria affects less frequently long bones such as the tibia, femur, and humerus [1]. Scarcely, the symptoms of osseous haemangioma in the extremities, such as localized swelling, limb hypertrophy, and local pain, are usually nonspecific. Hemorrhage can occur in the setting of trauma, biopsy, or surgery [4].

In our case, there was no osteolysis. The haemangioma is presented as a hair image with peripheral sclerosis. In most cases, haemangioma are presented as osteolysis. The osteolysis was unifocal and diaphyseal [2] [8]. Conventional radiography along with clinical and pathologic data can aid in diagnosing of the wide variety of disease processes that involve the tibial cortex [9].

Bone lysis can have linear and circular components on radiographs, suggestive of a vascular lesion, with linear and circular elements representing vascular channels seen longitudinally and in front respectively. However, these serpentine vascular channels are recognized more easily with CT and MR imaging [5] [8]. Honeycomb, soap bubble, or cystic appearance on radiographs or CT and markedly high-signal intensity on T2-weighted magnetic resonance images (MRIs) have a diagnostic value, but are not decisional for diagnosis [3] [7]. These images were found in our case. Characteristically, these channels have a low signal intensity on T1-weighted images and a very high signal intensity on T2-weighted images because of the slow blood flow. In arteriovenous lesions with a faster blood flow, a low signal intensity may persist with all MR imaging pulse sequences [10] [11].

At the tibia, periosteal or cortical haemangioma occurs most frequently in the anterior tibial diaphysis. These lytic cortical lesions may also show the characteristic multifocal vascular channels or be seen as a larger, nonspecific region of bone destruction. Cortical haemangiomas may predispose the bone to fracture, and periosteal reaction may accompany these lesions [9] [12].

A case of intraosseous arteriovenous malformation (AVM) arising in the diaphysis of the tibia was described by Matsuyama A. Plain radiographs displayed honeycomb lytic lesions in the right tibial shaft, with a slight expansion of the bone and cortical thinning. Curettage of the lesion was carried out, and the histological diagnosis of AVM was made [13].

The surgical treatment is recommended only if the patient is symptomatic or complicated by a pathological fracture [2].

The histopathological diagnosis of a haemangioma is not difficult in a whole sample. The diagnosis from biopsy or tissue curettage is a challenge. It reveals the destructive procedures disrupting thin-walled blood vessels and resulting in histological sections showing non diagnostic empty spaces with scattered bone trabeculae.

Histologically, haemangiomas can be classified as cavernous, capillary, venous or mixed, depending on the type of vascular involvement. Cavernous haemangioma is the most common type in the peripheral bones and accounts for up to 50% of all cases reported [8]. They are predominant in the medullary and the intracortical portion of the bone and are not yet reported in the diaphysal bone. Pure capillary haemangioma accounts for 10% of all these types as reported in the literature [11] [13].

Treatment of angiomas is size-dependent. Localized, well-circumscribed lesions are easily treated by cement injection. Larger lesions involving total bones are difficult to treat. Many different treatment options have been reported for haemangiomas of the bone involving the extremities, ranging from simple resection to radiation and even amputation [6] [8] [12].

Until recently, predominantly open excisional biopsy was performed, and therefore, surgical treatment was already complete once the definitive diagnosis was revealed by the histological workup [8].

Larger haemangioma in long bones are difficult to treat. If appropriate, the lesions should be excised and packed with bone graft. There are no reports in the literature of limb salvage when haemangioma occurs in nearly the whole long bone [10].

For Cristofaro R [14], selective arterial embolization represents a treatment of choice in aneurysmal bone angioma especially of the spine, sacrum, or pelvis. In these sites embolization replaces surgery which might be hazardous due to intraoperative bleeding. No recurrence was observed in any cases of his study.

There is controversy about the safety of ethanol injections in the treatment of vertebral haemangioma. The study of Bas T [15] shows that intralesional alcohol injections can be considered a safe technique for vertebral haemangiomas. However, a careful technique is required.

Sclerotherapy is the best therapeutic alternative in case of recurrent osseous haemangioma. Under CT guidance the affected bone is punctured by a biopsy needle. The sclerosant is then injected into the haemangioma. Sclerotherapy can be preceded by transcatheter embolization.

CT-guided intraosseous sclerotherapy is a successful therapy. The evolution is often marked by clinical and radiological improvement [16].

In some cases, the management of the haemangioma is made by the association of embolization, sclerotherapy, surgery and radiotherapy.

Cement injection has become a well-accepted treatment option for vertebral pathologies, such as angiomas [16]. The method seems to be well tolerated by the patient and can be done under local anaesthesia. Despite the anatomic proximity of neurologic, vascular structures and the variable high rate of cement extravasations (ranging from 20% up to 67%), the incidence of major complications is very low [1] [16].

4. Conclusion

Intramedullary veinous haemangioma of the leg is uncommon with Clinical symptoms mimicking an osteoid osteoma. MR imaging is considered the best mode of evaluating most soft-tissue masses, and haemangioma is no exception. For angiomatous lesions, MR imaging findings are frequently diagnostic. The histological examination confirms the diagnosis. Surgical excision does not prevent recurrence of lesion. Sclerothrapy guided by computed tomographic scan is a good alternative to stabilize intramedullary veinous haemangioma.

Conflict of Interest

There are no conflicts of interest.

References

[1] Kenan, S., Abdelwahab, I.F., Klein, M.J. and Lewis, M.M. (1992) Hemangiomas of the Long Tubular Bone. *Clinical Orthopaedics and Related Research*, No. 280, 256-260. http://dx.doi.org/10.1097/00003086-199207000-00035

[2] Ogose, A., Hotta, T., Morita, T., Takizawa, T., Ohsawa, H. and Harita, Y. (2000) Solitary Osseous Hemangioma outside the Spinal and the Craniofacial Bones. *Archives of Orthopaedic and Trauma Surgery*, **120**, 262-266. http://dx.doi.org/10.1007/s004020050461

[3] Sherman, R.S. and Wilner, D. (1961) The Roentgen Diagnosis of Hemangioma of Bone. *The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine*, **86**, 1146-1159.

[4] Resnik, D., Kyriakos, M. and Greenway, G.D. (2002) Tumours and Tumour-Like Lesions of Bone. In: Resnik, D, Ed., *Diagnosis of Bone and Joint Disorders*, 4th Edition, Saunders, Philadelphia, 3979-3985.

[5] Yamamoto, T., Kurosaka, M. and Mizuno, K. (2000) Juxta-Articular Hemangioma of Long Bone. *Skeletal Radiology*, **29**, 535-537. http://dx.doi.org/10.1007/s002560000256

[6] Thomas, A.M.C., Mulligan, P.J. and Jones, E.L. (1990) Benign Haemangioma of Bone in a Middle Phalanx. *The Journal of Hand Surgery*, **15**, 484-486. http://dx.doi.org/10.1016/0266-7681(90)90097-N

[7] Wenger, D.E. and Wold, L.E. (2000) Benign Vascular Lesions of Bone: Radiologic and Pathologic Features. *Skeletal Radiology*, **29**, 63-74. http://dx.doi.org/10.1007/s002560050012

[8] Kaleem, Z., Kyriakos, M. and Totty, W.G. (2000) Solitary Skeletal Hemangioma of the Extremities. *Skeletal Radiology*, **29**, 502-513. http://dx.doi.org/10.1007/s002560000251

[9] Levine, S.M., Lambiase, R.E. and Petchprapa, C.N. (2003) Cortical Lesions of the Tibia: Characteristic Appearances at Conventional Radiography. *RadioGraphics*, **23**, 157-177. http://dx.doi.org/10.1148/rg.231015088

[10] Yu, L., Cai, L., Yu, G.R., Zeng, Z.H. and Tao, S.X. (2009) Solitary Giant Hemangioma of the Humerus. *Orthopedics*, **32**, 925. http://dx.doi.org/10.3928/01477447-20091020-24

[11] Chawla, A., Singrakhia, M., Maheshwari, M., Modi, N. and Parmar, H. (2006) Intraosseous Haemangioma of the Proximal Femur: Imaging Findings. *British Journal of Radiology*, **79**, 64-66. http://dx.doi.org/10.1259/bjr/53131368

[12] Weiner, D. and Leeson, M.C. (1990) Hemangioma of the Tibia. *Orthopedics*, **13**, 1397-1400.

[13] Matsuyama, A., Aoki, T., Hisaoka, M., Yokoyama, K. and Hashimoto, H. (2008) A Case of Intraosseous Arteriovenous Malformation with Unusual Radiological Presentation of Low Blood Flow. *Pathology—Research and Practice*, **204**, 423-426. http://dx.doi.org/10.1016/j.prp.2008.01.006

[14] Cristofaro, R D., Biagini, R., Boriani, S., Ricci, S., Ruggieri, P. and Rossi, G. (1992) Selective Arterial Embolization in the Treatment of Aneurysmal Bone Cyst and Angioma of Bone. *Skeletal Radiology*, **21**, 523-527. http://dx.doi.org/10.1007/BF00195235

[15] Bas, T., Aparisi, F. and Bas, J.L. (2001) Efficacy and Safety of Ethanol Injections in 18 Cases of Vertebral Hemangioma. *Spine*, **26**, 1577-1582. http://dx.doi.org/10.1097/00007632-200107150-00015

[16] Cohen, J.E., Lylyk, P., Ceratto, R., Kaplan, L., Umansky, F. and Gomori, J.M. (2004) Percutaneous Vertebroplasty: Technique and Results in 192 Procedures. *Neurological Research*, **26**, 41-49. http://dx.doi.org/10.1179/016164104773026516

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Obesity in Hemodialysis Patients

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Abstract

Obesity is a cardiovascular risk factor in the general population. However, obesity on hemodialysis is associated with better survival. The aim of this study is to determine the prevalence of obesity in our chronic hemodialysis population and to specify the different cardiovascular complications. It is a cross-sectional study with 120 patients older than 18 years on hemodialysis. Abdominal obesity was defined as waist circumference > 94 cm in men and > 80 cm in women. The following were analyzed: socioeconomic and demographic parameters, lifestyle, initial renal disease, duration of hemodialysis, food consumption and body mass index (BMI), biological parameters (C-reactive protein (CRP), albumin, lipid profiles, serum calcium, phosphorus, parathyroid hormone), comorbidities: Diabetes, hypertension, stroke, coronary artery disease. Twenty-four patients had a BMI> 25. The prevalence of abdominal obesity was 20%. The sex Ratio was 0.71. The average age of our patients was 55.97 years [23 - 78 years]. Reduced physical activity was observed in 75% of patients. Duration in hemodialysis was 79 months. Hemoglobin (Hb) average was 8.9 g/dl, serum calcium was 2.25 mmol/l, serum phosphorus was 1.7 mmol/l, parathyroid hormone (PTH) was 412 pg/ml, albuminemia was 30.7 g/l, total cholesterol was 3.75 mmol/l and CRP was 15 U/l. Coronary artery disease was found in 20.8% of cases, stroke in 8.3% of cases, diabetes in 58.33% of cases and hypertension in 75% of cases. There was a high prevalence of abdominal obesity in hemodialysis patients. Obesity is recognized by its association with increased risk of cardiovascular disease and mortality. However in the paradoxical epidemiology, obesity becomes in chronic hemodialysis, a protective factor and is associated with better survival. Our study has a small population and cannot reach such a conclusion; further studies with larger numbers are needed to support this concept.

Keywords

Hemodialysis, Obesity, Metabolic Syndrome, Abdominal Fat

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1. Introduction

A significant number of patients who undergo dialysis are overweight or obese [1] [2]. The presence of excess weight, especially visceral obesity contributes to the increased risk of metabolic and cardiovascular complications in patients with chronic kidney disease (CKD). The relationship between weight and outcome is complex among dialysis patients. The extra weight is usually associated with improved survival in this patient group [3].

2. Patients and Methods

It is a cross-sectional study in the Hemodialysis Unit of the Nephrology Department of Medicine A at the Charles Nicolle Hospital in Tunis in January 2015.

Inclusion criteria: Chronic hemodialysis patients older than 18 years with abdominal obesity defined as waist circumference > 94 cm in men and > 80 cm in women [4].

Exclusion criteria: Patients on hemodialysis for less than 3 months and patients with acute infection, or active neoplasia.

Twenty-four patients were selected for this study. For each patient were analyzed: socioeconomic and demographic parameters, lifestyle, initial renal disease, duration of HD, food consumption and body mass index (BMI), biological parameters: C-reactive protein (CRP), albumin, lipid profiles, serum calcium, phosphorus, parathyroid hormone, comorbidities: Diabetes, hypertension (HT), stroke, coronary artery disease.

3. Results

The prevalence of abdominal obesity was 20%. Twenty-four patients had a BMI > 25. The mean waist circumference was 85 cm in women [80.5 - 95 cm] and 98 in men [94.8 - 102 cm]. The sex Ratio was 0.71. The average age of our patients was 55.97 years [23 - 78 years]. Reduced physical activity was observed in 75% of patients. The prevalence of smoking was 55%.

Duration in HD was 79 months. Seven patients had vascular nephropathy, 10 had diabetic nephropathy, 4 had chronic glomerular nephritis, 2 had chronic interstitial nephropathy and one patient had hereditary kidney disease (tuberous sclerosis of Bourneville). The average time between discovery of kidney failure and the onset of hemodialysis was 20 months.

Hemoglobin (Hb) average was 8.9 g/dl [7.9 - 9.8 g/dl], serum calcium was 2.25 mmol/l [2.08 - 2.55 mmol/l], serum phosphorus was 1.7 mmol/l [1.5 - 2.2 mmol/l]. Secondary hyperparathyroidism was found in 12 patients (50%) with a mean PTH of 412 pg/ml.

The mean albuminimia was 30.7 g/l [27 - 35 g/l] and the mean total cholesterol was 3.75 mmol/l [2.9 - 4 mmol/l].

The inflammatory condition was evaluated using CRP as a marker of inflammation. Sixteen patients (66.6%) had an inflammatory syndrome with a mean CRP of 15 U/l.

Cardiovascular complications noted included coronary artery disease in 20.8% of cases, stroke in 8.3% of cases, diabetes in 58.33% of cases and HT in 75% of cases.

Demographic, clinical and biological characteristics were summarized in **Table 1**.

4. Discussion

In the general population, abdominal obesity is characterized by chronic low grade inflammation with increased serum inflammatory cytokine levels; it is considered to be a risk factor for atherosclerosis, cardiovascular disease, and increased mortality [5].

In obese dialysis patients, the nutritional status may be better, and obesity provides, in the short term, some protection against malnutrition and the associated morbidity. However, some studies suggest that mortality in the long term is directly correlated with excess weight and obesity, which indicates that fat represents a risk factor also in uremia [1] [3]. Observational studies in CKD patients as well as dialysis patients, link abdominal fat with inflammation, insulin resistance, hyperadipokinemia, dyslipidemia, oxidative stress [6]-[9] and cardiovascular events and mortality [10]-[12].

In patients requiring dialysis, disturbances in lipid and carbohydrate metabolism, which are common, have been associated with abdominal fat and may develop into metabolic syndrome (MetS) [13]. There are multiple

Table 1. Demographic, clinical and biological characteristics.

Parameters	
Age (years)	55.97
Smoking	55%
Duration in hemodialysis (months)	79
Time between kidney failure and onset of HD (months)	20
Hemoglobin (g/dl)	8.9
Calcium (mmol/l)	2.25
Phosphorus (mmol/l)	1.7
PTH (pg/ml)	412
Albumin (mmol/l)	30.7
Cholesterol (mmol/l)	3.75
CRP (UI/L)	15

definitions of MetS, and the most current of MetS definitions is the National Cholesterol Education Program/ Adult Treatment Panel III [14]. It recognizes five components: hypertension, impaired fasting glucose, hypertriglyceridemia, abdominal obesity and low high-density lipoprotein cholesterol (HDL-C). The prevalence of MetS in chronic HD patients ranges from 40% - 60%; important relationship between MetS and the development of cardiovascular disease in chronic HD patients is found [15].

Recent evidence suggests that abdominal obesity play a role in peripheral arterial disease in dialysis patients [2]. Carotid artery intima media thickness is associated with waist circumference in peritoneal dialysis patients [16]. These vascular involvements are associated with morbidity in dialysis patients [17]. In our study, coronary artery disease was found in 20.8% of cases, stroke in 8.3% of cases, diabetes in 58.33% of cases and HT in 75% of cases.

Moreover, obesity does not necessarily imply good nutritional status. In patients with chronic kidney disease, there is a loss of muscle mass despite an excess of adipose tissue, which is a condition known as sarcopenic obesity [3] [6]. Malnutrition associated with obesity, including sarcopenic obesity, is the risk factor most closely correlated with morbidity and mortality both in dialysis patients and the general population [3].

BMI is inadequate as a measure of sarcopenic obesity since it cannot define neither muscle mass nor indicate the localization of the fat in the visceral compartment. Other indices must be developed and validated in well performed clinical trials to identify fat localization and the presence of sarcopenia [3].

Observational studies indicate improved survival in obese patients undergoing HD. Thus, it is hypothesized that dialysis patients at high risk of Protein-energy wasting (PEW) are protected by excess weight [18] [19]. The recent observations that waist circumference modifies the mortality risk associated with circulating triglycerides [20], leptin, and adiponectin [21], underscores the overall effect that abdominal obesity has on PEW.

Insulin resistance represents a major target for intervention in PEW. Alterations in glucose metabolism in the face of hyperinsulinemia and diminished tissue sensitivity to insulin are partially correctable by HD [18].

CKD patients have increased expression of proinflammatory cytokines and adipokines in abdominal subcutaneous tissue compared with healthy controls [22]-[24]. In our study, sixteen patients (66.6%) had an inflammatory syndrome. This high rate is explained mainly by oxidative stress. In addition, this inflammation is responsible for the low Hb levels (8.9 g/dl) in addition to the non-availability of recombinant erythropoietin in our hospital.

However, dialysis reverses uremia, residual metabolic derangements, inflammation, comorbid conditions, and the dialysis procedure itself may allow PEW to develop or worsen.

Leptin, and adiponectin, which were not dialyzable, were unfortunately not measured in our laboratory.

5. Conclusion

A good nutritional state is found in some obese dialysis patients, which probably accounts for the improved

survival of the obese group. The results of the present study showed that there was a high prevalence of abdominal obesity in hemodialysis patients but cannot reach such a conclusion. Therefore, further studies are needed to clarify the prognostic effect of abdominal obesity in dialysis patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Freitas, A.T., Vaz, I.M., Ferraz, S.F., Peixoto Mdo, R., Campos, M.I. and Fornés, N.S. (2013) Prevalence and Associated Factors with Abdominal Obesity in Hemodialysis Patients in Goiânia—GO. *Jornal Brasileiro de Nefrologia*, **35**, 265-272. http://dx.doi.org/10.5935/0101-2800.20130045

[2] Yang, S.Y., Chiang, C.K., Hsu, S.P., Peng, Y.S., Pai, M.F., Ho, T.I., *et al.* (2007) Metabolic Syndrome Predicts Hospitalization in Hemodialysis Patients: A Prospective Asian Cohort Study. *Blood Purification*, **25**, 252-259. http://dx.doi.org/10.1159/000101698

[3] Panzetta, G. and Abaterusso, C. (2010) Obesity in Dialysis and Reverse Epidemiology: True or False? *Giornale Italiano di Nefrologia*, **27**, 629-638.

[4] Furman-Niedziejko, A., Rostoff, P., Rychlak, R., Golinska-Grzybala, K., Wilczynska-Golonka, M., Golonka, M., *et al.* (2014) Relationship between Abdominal Obesity, Platelet Blood Count and Mean Platelet Volume in Patients with Metabolic Syndrome. *Folia Medica Cracoviensia*, **54**, 55-64.

[5] Berg, A.H. and Scherer, P.E. (2005) Adipose Tissue, Inflammation, and Cardiovascular Disease. *Circulation Research*, **96**, 939-949. http://dx.doi.org/10.1161/01.RES.0000163635.62927.34

[6] Honda, H., Qureshi, A.R., Axelsson, J., Heimburger, O., Suliman, M.E., Barany, P., *et al.* (2007) Obese Sarcopenia in Patients with End-Stage Renal Disease Is Associated with Inflammation and Increased Mortality. *The American Journal of Clinical Nutrition*, **86**, 633-638.

[7] Axelsson, J., Rashid Qureshi, A., Suliman, M.E., Honda, H., Pecoits-Filho, R., *et al.* (2004) Fat Mass as a Contributor to Inflammation in End-Stage Renal Disease. *The American Journal of Clinical Nutrition*, **80**, 1222-1229.

[8] Gohda, T., Gotoh, H., Tanimoto, M., Sato, M., Io, H., Kaneko, K., *et al.* (2008) Relationship between Abdominal Fat Accumulation and Insulin Resistance in Hemodialysis Patients. *Hypertension Research*, **31**, 83-88. http://dx.doi.org/10.1291/hypres.31.83

[9] Witasp, A., Carrero, J.J., Hammarqvist, F., Qureshi, A.R., Heimbürger, O., Schalling, M., *et al.* (2011) Expression of Osteoprotegerin in Human Fat Tissue; Implications for Chronic Kidney Disease. *European Journal of Clinical Investigation*, **4**, 498-506. http://dx.doi.org/10.1111/j.1365-2362.2010.02432.x

[10] Kamimura, M.A., Carrero, J.J., Canziani, M.E., Watanabe, R., Lemos, M.M. and Cuppari, L. (2013) Visceral Obesity Assessed by Computed Tomography Predicts Cardiovascular Events in Chronic Kidney Disease Patients. *Nutrition, Metabolism and Cardiovascular Diseases*, **23**, 891-897. http://dx.doi.org/10.1016/j.numecd.2012.06.004

[11] Postorino, M., Marino, C., Tripepi, G. and Zoccali, C., CREDIT (Calabria Registry of Dialysis and Transplantation) Working Group (2009) Abdominal Obesity and All-Cause and Cardiovascular Mortality in End-Stage Renal Disease. *Journal of the American College of Cardiology*, **53**, 1265-1272. http://dx.doi.org/10.1016/j.jacc.2008.12.040

[12] Wu, C.C., Liou, H.H., Su, P.F., Chang, M.Y., Wang, H.H., Chen, M.J., *et al.* (2011) Abdominal Obesity Is the Most Significant Metabolic Syndrome Component Predictive of Cardiovascular Events in Chronic Hemodialysis Patients. *Nephrology Dialysis Transplantation*, **26**, 3689-3695. http://dx.doi.org/10.1093/ndt/gfr057

[13] Huang, J.W., Yang, C.Y., Wu, H.Y., Liu, K.L., Su, C.T., Wu, C.K., *et al.* (2013) Metabolic Syndrome and Abdominal Fat Are Associated with Inflammation, But Not with Clinical Outcomes, in Peritoneal Dialysis Patients. *Cardiovascular Diabetology*, **12**, 86. http://dx.doi.org/10.1186/1475-2840-12-86

[14] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*, **285**, 2486-2497. http://dx.doi.org/10.1001/jama.285.19.2486

[15] Ucar, E., Huzmeli, C., Guven, O., Savas, N., Gullu, M., Asilyoruk, S., *et al.* (2009) Frequency of Metabolic Syndrome among Hemodialysis Patients According to NCEP-ATP III and IDF Definitions. *Ren Fail*, **31**, 221-228. http://dx.doi.org/10.1080/08860220802669883

[16] Asicioglu, E., Kahveci, A., Arikan, H., Koc, M., Tuglular, S. and Ozener, C.I. (2013) Waist Circumference Is Associated with Carotid Intima Media Thickness in Peritoneal Dialysis Patients. *International Urology and Nephrology*, **45**, 1437-1443. http://dx.doi.org/10.1007/s11255-013-0427-x

[17] Hung, P.H., Tsai, H.B., Lin, C.H. and Hung, K.Y. (2013) Abdominal Obesity Is Associated with Peripheral Artery Disease in Hemodialysis Patients. *PLoS One*, **8**, e67555. http://dx.doi.org/10.1371/journal.pone.0067555

[18] Carrero, J.J., Stenvinkel, P., Cuppari, L., Ikizler, T.A., Kalantar-Zadeh, K., Kaysen, G., *et al*. (2013) Etiology of the Protein-Energy Wasting Syndrome in Chronic Kidney Disease: A Consensus Statement from the International Society of Renal Nutrition and Metabolism (ISRNM). *Journal of Renal Nutrition*, **23**, 77-90. http://dx.doi.org/10.1053/j.jrn.2013.01.001

[19] Fouque, D., Kalantar-Zadeh, K., Kopple, J., Cano, N., Chauveau, P., Cuppari, L., *et al.* (2008) A Proposed Nomenclature and Diagnostic Criteria for Protein-Energy Wasting in Acute and Chronic Kidney Disease. *Kidney International*, **73**, 391-398. http://dx.doi.org/10.1038/sj.ki.5002585

[20] Postorino, M., Marino, C., Tripepi, G. and Zoccali, C. (2011) Abdominal Obesity Modifies the Risk of Hypertriglyceridemia for All-Cause and Cardiovascular Mortality in Hemodialysis Patients. *Kidney International*, **79**, 765-772. http://dx.doi.org/10.1038/ki.2010.493

[21] Zoccali, C., Postorino, M., Marino, C., Pizzini, P., Cutrupi, S. and Tripepi, G. (2011) Waist Circumference Modifies the Relationship between the Adipose Tissue Cytokines Leptin and Adiponectin and All-Cause and Cardiovascular Mortality in Haemodialysis Patients. *Journal of Internal Medicine*, **269**, 172-181. http://dx.doi.org/10.1111/j.1365-2796.2010.02288.x

[22] Carrero, J.J., Cordeiro, A.C., Lindholm, B. and Stenvinkel, P. (2010) The Emerging Pleiotrophic Role of Adipokines in the Uremic Phenotype. *Current Opinion in Nephrology & Hypertension*, **19**, 37-42. http://dx.doi.org/10.1097/MNH.0b013e328332fc2b

[23] Witasp, A., Carrero, J.J., Heimbürger, O., Lindholm, B., Hammarqvist, F., Stenvinkel, P., *et al*. (2011) Increased Expression of Pro-Inflammatory Genes in Abdominal Subcutaneous Fat in Advanced Chronic Kidney Disease Patients. *Journal of Internal Medicine*, **269**, 410-419. http://dx.doi.org/10.1111/j.1365-2796.2010.02293.x

[24] Carvalho, L.K., Silva, M.I.B., da Silva Vale, B., Bregman, R., Martucci, R.B., Carrero, J.J. and Avesani, C.M. (2012) Annual Variation in Body Fat Is Associated with Systemic Inflammation in Chronic Kidney Disease Patients Stages 3 and 4: A Longitudinal Study. *Nephrology Dialysis Transplantation*, **27**, 1423-1428. http://dx.doi.org/10.1093/ndt/gfr450

Abbreviations

Body mass index: BMI
C-reactive protein: CRP
Chronic kidney disease: CKD
Hemodialysis: HD
Hemoglobin: Hb
Hypertension: HT
Metabolic syndrome: MetS
Parathyroid hormone: PTH
Protein-energy wasting: PEW

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Sitagliptin for Elderly Patients Aged 75 Years or Older with Inadequately Controlled Type 2 Diabetes with Common Antidiabetes Treatments

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Abstract

Background: The purpose of this study was to evaluate the effects of sitagliptin in elderly patients with type 2 diabetes aged 75 years or older versus those aged 65 - 74 years. Methods: Outpatients aged 65 years or older with type 2 diabetes who received sitagliptin at a dose of 50 mg daily for 6 months were divided into two groups: those who were 75 years and older and those who were 65 - 74 years. The efficacy and safety were retrospectively evaluated by comparison of laboratory values before and after the administration of sitagliptin and by review of adverse events after treatment. Results: One hundred and twelve older patients with type 2 diabetes were studied. Six months after the initiation of sitagliptin, participants' hemoglobin A1c was significantly decreased by 1.09% ± 0.8% in 65 - 74-year-olds (66 patients; mean age, 69.1 ± 3.0 years; mean HbA1c before administration, 8.4% ± 0.8%) and by 1.05% ± 0.8% in patients 75 years or older (46 patients; mean age, 79.8 ± 4.1 years; mean HbA1c before administration, 8.5% ± 0.7%). There was no significant difference in hemoglobin A1c between the two groups. Furthermore, sitagliptin was well tolerated in both age groups. Conclusions: In elderly patients (75 years or older) with type 2 diabetes, the effect of sitagliptin was similar that in older patients (younger than 75 years) with type 2 diabetes.

Keywords

Elderly, Sitagliptin, Type 2 Diabetes Mellitus

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1. Introduction

According to the National Health and Nutrition Survey in Japan conducted in 2012, the number of people "strongly suspected to have diabetes" is rising in Japan. Those 65 years and older accounted for more than half of the patients studied [1]. Moreover, as the population ages, the number of patients aged 75 years or older with diabetes will also increase. However, an analysis [2] of large-scale clinical trials showed that only 0.6% of interventional trials in diabetes treatment specifically targeted this age group; 31% excluded patients older than 65; and almost all excluded those older than 75. Treatment of elderly patients with type 2 diabetes is challenging because of the high prevalence of comorbidities, use of polypharmacy, frailty, and the age-related reduction in pancreatic islet cell function [3]. Safety is therefore an important consideration for treatment, especially the avoidance of iatrogenic hypoglycemia which occurs frequently in elderly patients and can have severe consequences [4] [5]. Renal impairment is also very common in elderly patients with type 2 diabetes, which increases their risk for hypoglycemia and complicates treatment strategies [6]. The management of elderly patients with type 2 diabetes is further complicated by its clinical heterogeneity, *i.e.*, very different disease durations and degrees of frailty, as well as the very limited availability of clinical trial data, especially in the very elderly subgroup [7].

The dipeptidyl peptidase-4 (DPP4) inhibitors, a new class of oral antidiabetic agents, could be particularly promising for the treatment of older patients [8] [9]. Sitagliptin, a member of this class, has been studied in a large clinical program, showing good overall safety and tolerability and improving glycemic control when given as monotherapy or combination therapy [10]-[14]. A very low incidence of hypoglycemia has consistently been reported with sitagliptin across the disease spectrum [15]-[20]. In addition, we consider that sitagliptin is effective for the treatment of type 2 diabetes in elderly patients because it is taken once daily and therefore adherence to the treatment is favorable.

The aim of this study was to investigate the glucose-lowering efficacy, safety, and tolerability of sitagliptin in patients aged 65 - 74 years versus those 75 years and older with type 2 diabetes whose glycemic control was inadequate on other common glucose-lowering drugs.

2. Methods

2.1. Study Design and Patients

Among patients aged 65 years and older with type 2 diabetes being treated at our diabetes clinic on an outpatient basis, those who received sitagliptin once daily at a dose of 50 mg (25 mg when estimated glomerular filtration rate [eGFR] was below 30 mL/min per 1.73 m^2), and who were treated for at least 6 months were examined. Changes in hemoglobin A1c (HbA1c) before and after treatment with sitagliptin were retrospectively analyzed. The patients were divided into two groups: those 75 years or older and those 65 - 74 years. The difference in the effect of sitagliptin between these two groups was assessed. The observation period was from January 2010 to January 2014. Medical records were examined for dosages administered and adverse events (AEs). Exclusion criteria included add on other antidiabetic agents or drugs affecting plasma glucose or changes in the previously use of other antidiabetic agents and in instructions for diet or exercise therapy during the study period.

2.2. Assessments

HbA1c, body weight, sitting systolic and diastolic blood pressure and common laboratory parameters were measured periodically during the study period. All adverse events (AEs) were recorded and assessed by the investigator with regard to severity and possible relationship to the study medication.

Glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) method and data in the group of patients with severe renal impairment (eGFR < 30 mL/min/1.73 m^2) are presented (**Table 1**). Confirmed hypoglycemia was defined as symptoms suggestive of low blood glucose confirmed by self-monitored blood glucose (SMBG) measurement <3.1 mmol/L plasma glucose equivalent. Severe hypoglycemia was defined as any episode requiring the assistance of another party.

2.3. Data Analysis

The data were summarized by mean values and standard deviations. Statistical analysis was performed using

Table 1. Baseline characteristics by age groups.

	65 - 74 y (n = 66)	≥75 y (n = 46)	*P*-value
Age (y)	69.1 ± 3.0	79.8 ± 4.1	<0.001
Male, No. (%)	38 (57.5)	27 (58.6)	0.999
Body weight (kg)	61.3 ± 10.8	58.0 ± 10.2	0.196
BMI (kg/m^2)	24.3 ± 3.8	23.8 ± 3.3	0.453
Duration of Diabetes (y)	14.4 ± 7.3	16.1 ± 8.8	0.274
HbAc1 (%)	8.43 ± 0.8	8.49 ± 0.7	0.725
SBP (mmHg)	126.5 ± 12.5	123.8 ± 9.8	0.223
DBP (mmHg)	72.9 ± 9.9	69.0 ± 10.3	0.051
eGFR (ml/min/1.73 m^2)	74.6 ± 22.2	67.0 ± 19.3	0.068
Complication[†]			
Hypertension	41 (62.1)	34 (73.0)	0.224
Dyslipidemia	32 (48.4)	19 (41.3)	0.563
Chronic Heart failure	5 (7.5)	1 (2.1)	0.398
OMI	5 (7.7)	8 (17.3)	0.138
AF	1 (1.5)	2 (4.3)	0.567
ASO	1 (1.5)	2 (4.3)	0.567
Stroke	1 (1.5)	0 (0.0)	0.999
Other OHA[‡]			
Metformin	47 (71.2)	22 (47.8)	0.018
SU	31 (46.9)	27 (58.7)	0.252
Pioglitazone	6 (9.0)	7 (15.2)	0.376
αGI	2 (3.0)	2 (4.3)	0.999
Insulin	15 (22.7)	13 (28.2)	0.515

Unless indicated otherwise, data are shown as the mean ± SD. Values show the difference compared between two groups (two-tailed Student's paired or unpaired t-test and Fisher's exact test). [†]All complications are expressed as No. (%); [‡]Oral hypoglycemic agents expressed as No. (%); Abbreviations: BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; OMI: old myocardial infarction; AF: atrial fibrillation; ASO: arteriosclerosis obliterans; OHA: oral hypoglycemic agents; SU: sulfonylurea; αGI: α-glucosidase inhibitor.

StatView version 5.0 for Windows (SAS Institute, City, NC, USA). The significance of differences between discrete variable data was analyzed by Fisher's exact test as appropriate. Differences between two variables were evaluated by two-tailed Student's paired or unpaired t-test as appropriate. A two-sided significance level of 5% was used. All HbA1c values are shown as National Glycohemoglobin Standardization Program values.

2.4. Ethics and Good Clinical Practice

All study participants provided written informed consent. This study was conducted according to the principals expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after full explanation of the purpose, nature, and risks of all procedures used. The study protocol was approved by the ethical review committee of Saiseikai Niigata Daini Hospital.

3. Results

As a result of screening during the above observation period, 142 patients were recruited for the study. Among them, 30 patients were excluded for the reasons mentioned in the study design section, and 112 patients with type 2 diabetes (65 men and 47 women; mean age, 73.5 ± 6.3 years; BMI, 24.1 ± 3.6 kg/m^2; disease duration, 15.1 ± 8.0 years; baseline HbA1c, 8.4% ± 0.8%) were included in the study (**Figure 1**). After 6 months of treatment with sitagliptin, a significant decrease in HbA1c, by 1.07% ± 0.8% ($P < 0.001$), was observed in all patients. **Table 1** shows the characteristics for patients 65 - 74 years old and those 75 years or older. The former group consisted of 66 patients. The mean age was 69.1 ± 3.0 years old (range, 65 - 74). Baseline HbA1c was 8.4 ± 0.8%. The latter group consisted of 46 patients. The mean age was 79.8 ± 4.1 years old (range, 75 - 92). Baseline HbA1c was 8.5% ± 0.7%. The proportion of patients who used metformin at baseline was significantly lower in the latter group. **Figure 2** shows changes in HbA1c in the two groups. Both groups demonstrated a significant decrease 3 months after the start of treatment with sitagliptin, which persisted up to 6 months. Changes

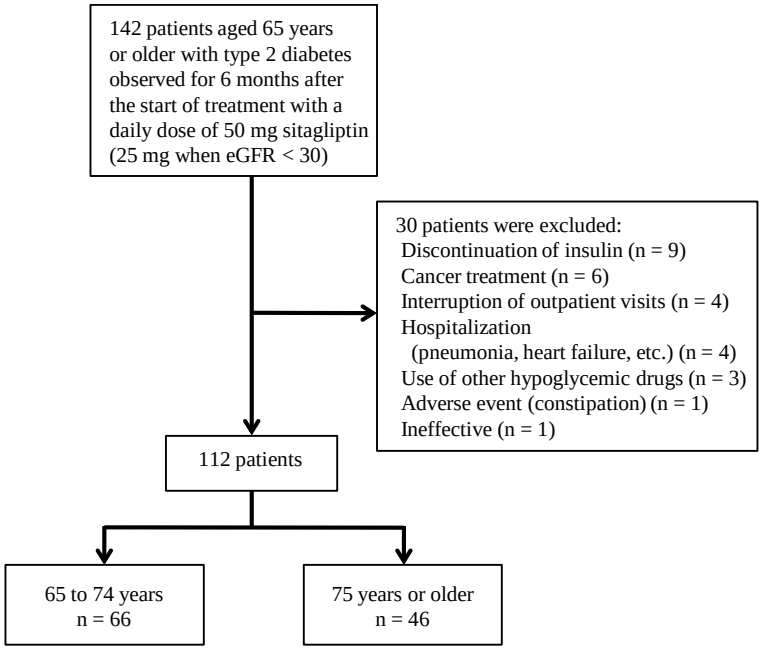
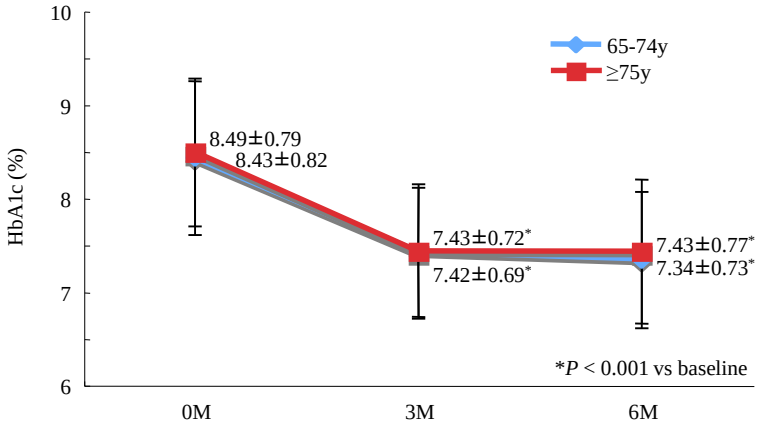


Figure 1. Trial profile.

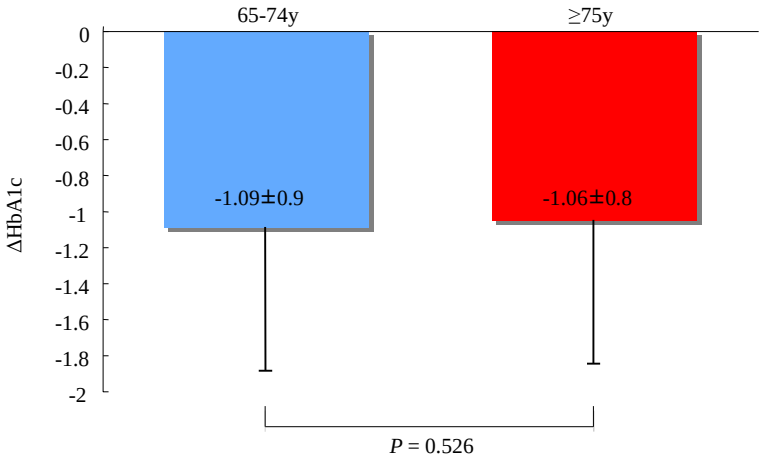


Data are showed as the mean ± SD. *p < 0.001 compared with baseline. No significant difference was observed between the two groups at baseline, 3 months and 6 months.

Figure 2. Change in the HbA1c levels from baseline at six-month intervals.

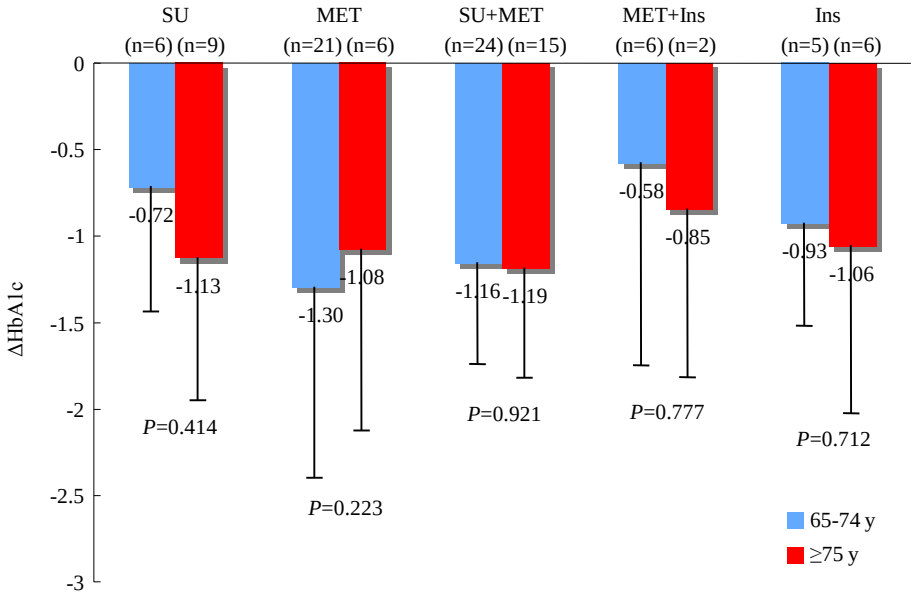
in HbA1c were thus similar among the two groups represented by a decrease of 1.09% ± 0.8% and 1.05% ± 0.8% for the 65 - 74-year-old and the 75 years or older groups, respectively, indicating a significant decrease from baseline in each group. No significant difference was observed between the two groups (**Figure 3**).

The change in HbA1c in relation to previous hypoglycemic agents used at baseline is shown in **Figure 4**. HbA1c was compared for patients previously treated with the following concomitant drugs: sulfonylurea (SU) alone; metformin alone; both SU and metformin; metformin and insulin; and insulin alone. HbA1c decreased significantly in all groups and no significant difference was observed between the two age groups. Next, change in HbA1c is presented versus the duration of diabetes in **Figure 5**. HbA1c was compared based on duration of disease: less than 5 years; 5 to 14 years; and 15 years or longer. HbA1c was significantly decreased in all groups. No significant difference was detected between the two age groups. **Figure 6** shows the change in HbA1c versus



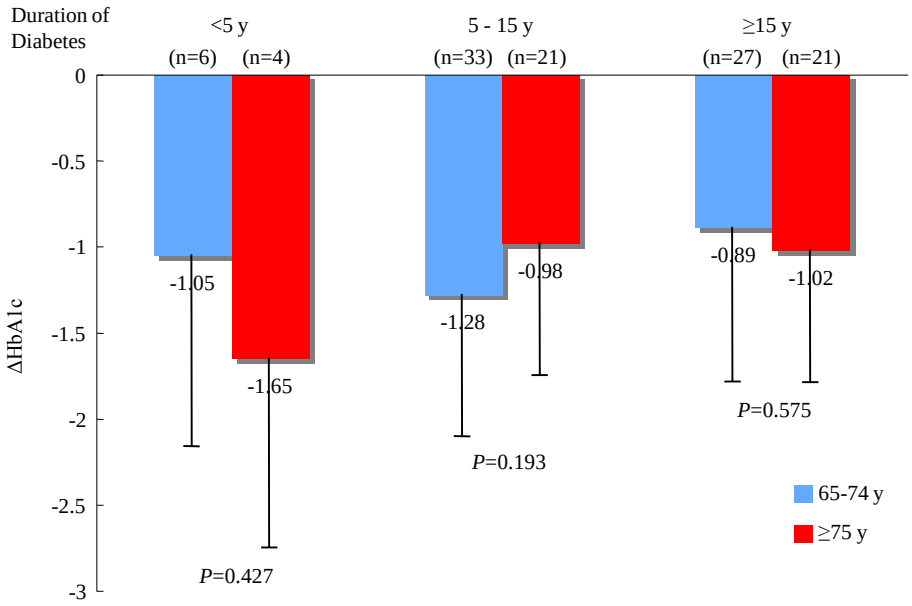
Data are showed as the mean ± SD.
No significant difference was observed between the two groups.

Figure 3. Adjusted mean changes in HbA1c from baseline to six months.



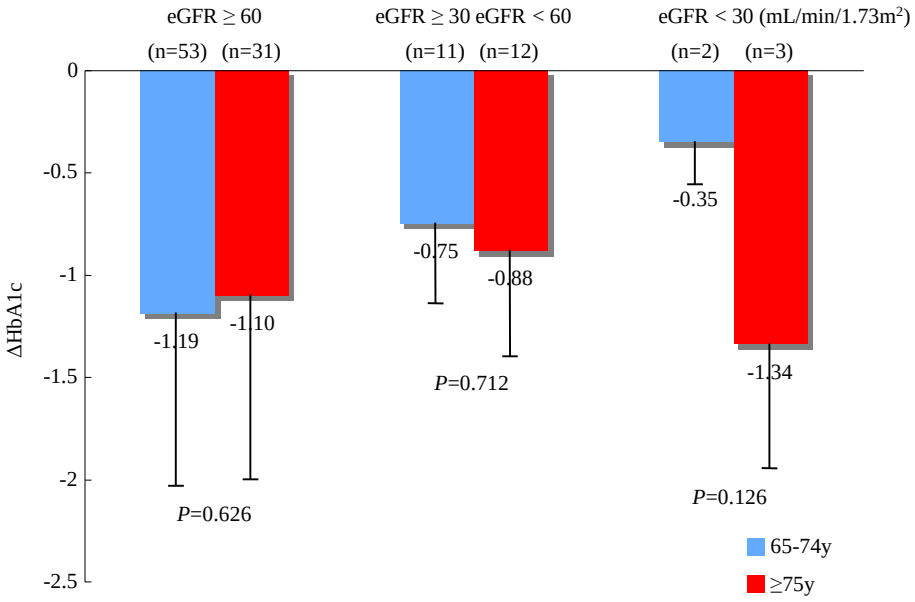
Data are showed as the mean ± SD. HbA1c decreased significantly in all groups and no significant difference was observed between the two age groups. SU: sulfonylurea alone; MET: metformin alone; SU+MET: both SU and metformin; MET+INS: metformin and insulin; and Ins: insulin alone.

Figure 4. Change in HbA1c versus previous therapeutic agent at baseline.



Data are showed as the mean ± SD. HbA1c decreased significantly in all groups and no significant difference was observed between the two age groups.

Figure 5. Decrease in HbA1c six months after addition of sitagliptin versus duration of diabetes.



Data are showed as the mean ± SD. HbA1c decreased significantly in all groups and no significant difference was observed between the two age groups. eGFR: estimated glomerular filtration rate;

Figure 6. Change in HbA1c versus baseline eGFR.

baseline eGFR. HbA1c was examined for patients whose eGFR was <30 mL/min/1.73 m^2, patients whose eGFR was ≥30 mL/min/1.73 m^2 and <60 mL/min/1.73 m^2, and patients whose eGFR was >60 mL/min/1.73 m^2. In all groups, HbA1c decreased significantly. There was no significant difference between the two age groups.

Table 2 compares changes in body weight and blood pressure between the two groups. Body weight and systolic blood pressure did not significantly change in either group. In patients aged 75 years or older, diastolic

Table 2. Changes in body weight and systolic and diastolic blood pressure in the two groups.

BW (kg)	0 Months	3 Months	6 Months	Adjusted mean change from baseline at 6 Months	*P*-value at 6 M vs. Baseline
65 - 74y	61.3 ± 10.9	61.3 ± 10.9	61.3 ± 11.0	0.024 ± 0.00	0.916
≥75y	58.0 ± 10.2	58.1 ± 10.2	58.2 ± 10.4	0.132 ± 0.00	0.622

P = 0.124 between two groups.

SBP (mmHg)	0 Months	6 Months	Adjusted mean change from baseline at 6 Months	*P*-value at 6 M vs. Baseline
65 - 74y	126.5 ± 12.4	126.1 ± 14.3	−0.409 ± 11.6	0.766
≥75y	123.8 ± 9.83	123.8 ± 12.2	0.00 ± 10.6	1.000

P = 0.376 between two groups.

DBP (mmHg)	0 Months	6 Months	Adjusted mean change from baseline at 6 Months	*P*-value at 6 M vs. Baseline
65 - 74y	72.9 ± 9.9	72.9 ± 10.9	−0.03 ± 11.0	0.982
≥75y	69.1 ± 10.3	66.9 ± 12.1	−2.10 ± 13.5	0.296

P = 0.008 between two groups.

Data are shown as the mean ± SD. Values show the difference compared between two groups. (two-tailed Student's paired or unpaired t-test). BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure.

blood pressure was slightly lowered and showed a significant difference at 6 months from the values in patients younger than 75 years.

There were few AEs. Constipation was observed in 2 patients (1 in each age group) and mild hypoglycemia in 2 patients (a patient treated with SU in those younger than 75 and a patient treated with insulin in those 75 years or older). No serious AEs were observed in either group. Sitagliptin was well tolerated.

4. Discussion

The present study demonstrates that, in elderly patients aged 75 years or older with type 2 diabetes whose plasma glucose had been insufficiently controlled by other hypoglycemic agents, sitagliptin improved plasma glucose level as effectively as in those patients aged 65 - 74 with poorly controlled type 2 diabetes. Furthermore, the drug was well tolerated. Review of the literature demonstrates that patients aged 75 years or older with type 2 diabetes have generally been excluded from clinical trials, even though they account for a large and growing proportion of patients with type 2 diabetes.

Perhaps one reason for the exclusion from such trials is concern about the risk of hypoglycemia, which poses a problem in drug therapy in older patients with diabetes. Hypoglycemia not only increases the risk of falls and fractures, but also possibly causes impairment of cognitive function [21] [22]. Authors of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial pointed out the relationship between severe hypoglycemia and higher mortality, specifically cardiovascular deaths, seen significantly more frequently in the intensive glucose control group than in the standard glucose control group [23]. This highlights the importance of prevention of hypoglycemia. The reasons for frequent hypoglycemia in elderly patients include: 1) slowed metabolism and excretion of drugs related to aging, 2) impairment of autonomic function, 3) decreased or unstable glucose intake, and 4) poor adherence to treatment due to impaired cognitive function.

The most important age-related change in pharmacokinetics occurs in the kidney. The weight of the kidney decreases with aging. Renal blood flow is speculated to be reduced by 1 mL/minute/year after the age of 30, resulting in a linear age-related decrease in eGFR starting in one's 40s. Additionally, many drugs are mainly metabolized in the liver. With aging, the weight of the liver also decreases and its metabolic capacity is reduced. These age-related changes delay the metabolism and excretion of drugs and result in an increased concentration

of drugs in the blood, which can lead to significant decreases in plasma glucose as well as other adverse drug reactions. Moreover, impairment of autonomic function increases the risk for sudden dysfunction of the central nervous system, which is responsible for subjective symptoms that warn of decreases in plasma glucose level, rendering the patient unaware of hypoglycemia. Thus, hypoglycemia is more likely to become severe. Furthermore, glucose intake may be greatly reduced due to seasonal changes and changes in environment for many elderly patients. In such cases, hypoglycemia can easily occur in patients treated with SUs, unless they are told how to prevent it. Impairment of cognitive function increases the risk of duplicate dosing of drugs as well as errors in choosing the correct insulin formulation and administering the correct dosage. Iwakura *et al*. raised advanced age, impaired renal function, use of SUs and insulin, and low HbA1c as factors that increase the risk of severe hypoglycemia [24].

Because oral hypoglycemic agents directly affect glycemic control, the prescription instructions need to be as simple as possible in order to improve adherence to the treatment. When glycemic control is gradually worsened, prescribing physicians should remember to confirm whether or not these drugs are being taken properly before increasing the dose or adding new oral hypoglycemic agents. It is often difficult to confirm this by talking only with patients. Discussion with family members is also important.

DPP-4 inhibitors are classified as insulin secretagogues. However, they promote insulin secretion only when plasma glucose level is high, and therefore, they do not frequently cause hypoglycemia when used as oral monotherapy. This is the greatest advantage of these drugs for use in elderly patients. However, the risk of hypoglycemia is increased in concomitant use with other drugs, especially SUs or insulin. In addition, DPP-4 inhibitors are taken once or twice a day, which is expected to help maintain adherence to the treatment. When renal function is impaired, these drugs can be used by adjusting the dose (although dose adjustment is unnecessary for linagliptin and teneligliptin). Thus, we speculate that these drugs can be used relatively safely in elderly patients. Among DPP-4 inhibitors, the outcome of administration of vildagliptin and linagliptin has been, in particular, examined in more elderly subjects with diabetes than others. A meta-analysis of studies on vildagliptin showed that the improvement in HbA1c as a result of using this drug was comparable in patients aged 65 years or older and patients younger than 65 years. Fasting plasma glucose and body weight were significantly and markedly decreased in the group of patients aged 65 years or older [25]. Moreover, Schweizer *et al*. evaluated this drug in even older patients, those aged 75 years or older (mean age, 76.9 years; range, 75 - 84 years) and confirmed it to be effective and well tolerated [26]. The subjects of the present study were even older than those of Schweizer and colleagues, being 79.8 years old on average, ranging from 75 - 92 years old. The study by Schweizer *et al*. was a meta-analysis of Phase II and III clinical studies, and most participants were healthy enough to be eligible for participation in such clinical studies. In contrast, our subjects were recruited from clinical practice, and therefore, we consider our data to be a better reflection of actual treatment. As far as we know, the present study is the first report showing that sitagliptin, the least examined DPP-4 inhibitor, in elderly patients with type 2 diabetes is as effective and as well tolerated as others in this class.

Linagliptin was shown to significantly decrease HbA1c by −0.64% compared with placebo and to be as safe and well tolerated as placebo in a study in which linagliptin or placebo was added to monotherapy with metformin, SU, or long-acting insulin, or to a combination of these drugs prescribed to patients aged 70 years or older [27]. Our study also demonstrates that sitagliptin is useful and safe when it is added to treatment with other hypoglycemic agents. Our study further shows its efficacy in elderly patients with diabetes aged 75 years or older with any renal dysfunction.

One major limitation of the present study is its retrospective design and the limited number of patients studied. Almost all of the patients received add-on therapy; the number of drug naïve patients was limited (n = 9). In addition, the duration of the study may be another limitation because the extent of the glycemic response may not have been fully elucidated over this time frame. Furthermore, other markers, such as markers for assessment of β-cell function in homeostatic models, and basal active GLP-1 levels, which could have contributed to the HbA1c reduction with sitagliptin, were not measured in the present study. Therefore, increased numbers of subjects and more detailed clinical parameters are needed in the future to avoid inappropriate extrapolation of the data.

The present study shows that sitagliptin added to existing glucose-lowering drugs is well tolerated, weight-neutral, and improves glycemic control in patients with type 2 diabetes aged 75 years or older (with characteristics typical of those encountered in clinical practice), similar to those less than 75 years old, without increasing the risk of AEs.

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References

[1] Japan Ministry of Health, Labor and Welfare: The 2012 National Health and Nutrition Survey in Japan. http://www.mhlw.go.jp/stf/houdou/0000032074.html

[2] Lakey, W.C., Barnard, K., Batch, B.C., Chiwell, K., Tasneem, A. and Green, J.B. (2013) Are Current Clinical Trials in Diabetes Addressing Importance Issues in Diabetes Care? *Diabetologia*, **56**, 1226-1235. http://dx.doi.org/10.1007/s00125-013-2890-4

[3] Fravel, M.A., McDanel, D.L., Ross, M.B., Moores, K.G. and Starry, M.J. (2011) Special Considerations for Treatment of Type 2 Diabetes Mellitus in the Elderly. *American Journal of Health-System Pharmacy*, **68**, 500-509. http://dx.doi.org/10.2146/ajhp080085

[4] Kirkman, M.S., Briscoe, V.J., Clark, N., *et al.* (2012) Diabetes in Older Adults. *Diabetes Care*, **35**, 2650-2664. http://dx.doi.org/10.2337/dc12-1801

[5] Sinclair, A., Morley, J.E., Rodriguez-Mañas, L., *et al.* (2012) Diabetes Mellitus in Older People: Position Statement on Behalf of the International Association of Gerontology and Geriatrics (IAGG), the European Diabetes Working Party for Older eople (EDWPOP), and the International Task Force of Experts in Diabetes. *Journal of the American Medical Directors Association*, **13**, 497-502. http://dx.doi.org/10.1016/j.jamda.2012.04.012

[6] Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., *et al.*, the American Diabetes Association (ADA), and the European Association for the Study of Diabetes (EASD) (2012) Management of Hyperglycemia in Type 2 Diabetes: A Patient-Centered Approach: Position Statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*, **35**, 1364-1379. http://dx.doi.org/10.2337/dc12-0413

[7] Halimi, S., Raccah, D., Schweizer, A. and Dejager, S. (2010) Role of Vildagliptin in Managing Type 2 Diabetes Mellitus in the Elderly. *Current Medical Research and Opinion*, **26**, 1647-1656. http://dx.doi.org/10.1185/03007995.2010.485881

[8] Mathieu, C. and Bollaerts, K. (2007) Antihyperglycaemic Therapy in Elderly Patients with Type 2 Diabetes: Potential Role of Incretinmimetics and DPP-4 Inhibitors. *International Journal of Clinical Practice*, **154**, 29-37. http://dx.doi.org/10.1111/j.1742-1241.2007.01437.x

[9] Abbatecola, A.M., Maggi, S. and Paolisso, G. (2008) New Approaches to Treating Type 2 Diabetes Mellitus in the Elderly: Role of Incretin Therapies. *Drugs Aging*, **25**, 913-925. http://dx.doi.org/10.2165/0002512-200825110-00002

[10] Maeda, H., Kubota, A., Tanaka, Y., Terauchi, Y. and Matsuba, I. (2012) The Safety, Efficacy and Predictors for HbA1c Reduction of Sitagliptin in the Treatment of Japanese Type 2 Diabetes. *Diabetes Research and Clinical Practice*, **95**, e20-e22. http://dx.doi.org/10.1016/j.diabres.2011.10.011

[11] Kubota, A., Maeda, H., Kanamori, A., Matoba, K., Jin, Y., Minagawa, F., Obana, M., *et al.* (2012) Efficacy and Safety of Sitagliptin Monotherapy and Combination Therapy in Japanese Type 2 Diabetes Patients. *Journal of Diabetes Investigation*, **3**, 503-509. http://dx.doi.org/10.1111/j.2040-1124.2012.00221.x

[12] Kubota, A., Matsuba, I., Saito, T., Nabe, K. and Seino, Y. (2011) Secretory Units of Islets in Transplantation Index Is a Useful Clinical Marker to Evaluate the Efficacy of Sitagliptin in Treatment of Type 2 Diabetes Mellitus. *Journal of Diabetes Investigation*, **2**, 377-380. http://dx.doi.org/10.1111/j.2040-1124.2011.00109.x

[13] Kubota, A., Maeda, H., Kanamori, A., Matoba, K., Jin, Y., Minagawa, F., Obana, M., *et al.* (2012) Pleiotropic Effects of Sitagliptin in the Treatment of Type 2 Diabetes Mellitus Patients. *Journal of Clinical Medicine Research*, **4**, 309-313. http://dx.doi.org/10.4021/jocmr1061w

[14] Chung, H.S. and Lee, M.K. (2011) Efficacy of Sitagliptin When Added to Ongoing Therapy in Korean Subjects with Type 2 Diabetes Mellitus. *Diabetes & Metabolism Journal*, **35**, 411-417. http://dx.doi.org/10.4093/dmj.2011.35.4.411

[15] Nonaka, K., Kakikawa, T., Sato, A., Okuyama, K., Fujimoto, G., Kato, N., Suzuki, H., *et al.* (2008) Efficacy and Safety of Sitagliptin Monotherapy in Japanese Patients with Type 2 Diabetes. *Diabetes Research and Clinical Practice*, **79**, 291-298. http://dx.doi.org/10.1016/j.diabres.2007.08.021

[16] Tajima, N., Kadowaki, T., Odawara, M., Nishii, M., Taniguchi, T. and Arjona Ferreira, J.C. (2011) Addition of Sitagliptin to Ongoing Glimepiride Therapy in Japanese Patients with Type 2 Diabetes over 52 Weeks Leads to Improved Glycemic Control. *Diabetology International*, **2**, 32-44. http://dx.doi.org/10.1007/s13340-011-0022-2

[17] Kashiwagi, A., Kadowaki, T., Tajima, N., Nonaka, K., Taniguchi, T., Nishii, M., Arjona Ferreira, J.C. and Amatruda, J.M. (2011) Sitagliptin Added to Treatment with Ongoing Pioglitazone for Up to 52 Weeks Improves Glycemic Control in Japanese Patients with Type 2 Diabetes. *Journal of Diabetes Investigation*, **2**, 381-390.

http://dx.doi.org/10.1111/j.2040-1124.2011.00120.x

[18] Kadowaki, T., Tajima, N., Odawara, M., Nishii, M., Taniguchi, T. and Arjona Ferreira, J.C. (2013) Addition of Sitagliptin to Ongoing Metformin Monotherapy Improves Glycemic Control in Japanese Patients with Type 2 Diabetes over 52 Weeks. *Journal of Diabetes Investigation*, **42**, 174-181. http://dx.doi.org/10.1111/jdi.12001

[19] Tajima, N., Kadowaki, T., Okamoto, T., Sato, A., Okuyama, K., Minamide, T. and Arjona Ferreira, J.C. (2013) Sitagliptin Added to Voglibose Monotherapy Improves Glycemic Control in Patients with Type 2 Diabetes. *Journal of Diabetes Investigation*, **4**, 595-604.

[20] Kadowaki, T., Tajima, N., Odawara, M., Minamide, T., Kawashima, M., Yanagida, D., Okamoto, T. and Arjona Ferreira, J.C. (2013) Efficacy and Safety of Sitagliptin Add-On Therapy in Japanese Patients with Type 2 Diabetes on Insulin Monotherapy. *Diabetology International*, **4**, 160-172. http://dx.doi.org/10.1007/s13340-013-0109-z

[21] Johnston, S.S., Conner, C., *et al.* (2012) Association between Hypoglycaemic Events and Fall-Related Fractures in Medicare-Covered Patients with Type 2 Diabetes. *Diabetes, Obesity and Metabolism*, **14**, 634-643. http://dx.doi.org/10.1111/j.1463-1326.2012.01583.x

[22] Whitmer, R.A., Karter, A.J., *et al.* (2009) Hypoglycemic Episodes and Risk of Dementia in Older Patients with Type 2 Diabetes Mellitus. *JAMA*, **301**, 1565-1572. http://dx.doi.org/10.1001/jama.2009.460

[23] Bonds, D.E., Miller, M.E., *et al.* (2010) The Association between Symptomatic Severe Hypoglycemia and Mortality in Type 2 Diabetes: Retrospective Epidemiological Analysis of the ACCORD Study. *BMJ*, **340**, b4909. http://dx.doi.org/10.1136/bmj.b4909

[24] Iwakura, T., Sasaki, S., *et al.* (2012) Clinical Analysis of 135 Type 2 Diabetes Patients with Severe Drug-Induced Hypoglycemia. *Journal of the Japan Diabetes Society*, **55**, 857-865.

[25] Pratley, R.E., Rosenstock, J., *et al.* (2007) Management of Type 2 Diabetes in Treat-Naïve Elderly Patients: Benefits and Risks of Vildagliptin Monotherapy. *Diabetes Care*, **30**, 3017-3022. http://dx.doi.org/10.2337/dc07-1188

[26] Schweizer, A., Dejager, S., *et al.* (2011) Clinical Experience with Vidagliptin in the Management of Type 2 Diabetes in a Patient Population ≥75 Years: A Pooled Analysis from a Database of Clinical Trials. *Diabetes, Obesity and Metabolism*, **13**, 55-64. http://dx.doi.org/10.1111/j.1463-1326.2010.01325.x

[27] Barnett, A.H., Huisman, H., *et al.* (2013) Linagliptin for Patients Aged 70 Years or Older with Type 2 Diabetes Inadequately Controlled with Common Anti-Diabetes Treatments: A Randomized, Double-Blind, Placebo-Controlled Trials. *The Lancet*, **382**, 1413-1423. http://dx.doi.org/10.1016/S0140-6736(13)61500-7

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Role of Xuesaitong in Amelioration of Neural Function and Alteration of Bax Expression in Rats with Brain Trauma

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Abstract

Traumatic brain injury (TBI) is one of the most common diseases in clinical neurosurgery characterized with high incidence rate, mortality and many complications. Objectives: The purpose of this study is to explore the roles of Xuesaitong in the therapeutic effect of brain trauma and alteration of expression in Bax, a kind of promoting apoptosis factor. Methods: The rat traumatic brain injury models were established by using modified free falling body impact method. Thereafter, Xuesaitong was employed to be administered to TBI rats, and NSS Score Rating Scale was used to evaluate the effect of Xuesaitong. Moreover, real-time PCR was used to detect the Bax expression changes before and after the Xuesaitong administration. Results: Xuesaitong could accelerate the neurofunctional recovery of TBI rats, accompanied by NSS Scores significant decrease. Simultaneously, it also could inhibit the expression of Bax factor. Conclusions: Xusaitong could markedly ameliorate TBI restoration, in which it promotes the neurofunctional recovery, and at the same time it inhibits the expression of Bax.

Keywords

Traumatic Brain Injury, Neurofunction, Xuesaitong Injections, Bax

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1. Introduction

Traumatic Brain Injury (TBI) is one of the most common diseases in neurosurgery with high incidence rate, mortality and muti-complications. It could result in movement defect, cognition disorder, impairment of language function. The lesion of TBI also includes direct injury occurring at the injury site and secondary injury induced by the pathological process including ischemia, anoxia, calcium channel dysfunction and lipid peroxidation [1]. Among all the complications, cognition disorder is the most sustained and serious symptom, which expressed as juries or loss of attention and memory [1] [2]. While local brain ischemia is one of the main causes of secondary craniocerebral injury, 90% among which is the direct cause to death [3]. Currently, to our knowledge, there are several following methods dedicated to clinical therapy for TBI: cryotherapy, hormonal therapy, neurotrophic factors and growth factors therapies, cellular transplanting therapies (such as Bone Marrow Stem cells (BMSC) and Neural Stem Cells (NSCs)) [2].

The main component of Xuesaitong freeze-dry powder for injection is Panax Notoginsenosidum (PNS) extracted from Panax Notoginseng (Sanqi), which is a kind of famous traditional Chinese Medicine. It has various effects of activating blood circulation to dissipate blood stasis, promoting blood circulation and activating collaterals, so it can stretch blood vessels, inhibit the platelet aggregation, reduce the content of fibrinogen in the blood flow and blood viscosity, thereby ameliorate the microcirculation and exert pharmacologic actions of anti-inflammation and anti-oxidant [4]. All these accelerate the absorption of hematoma, promote the fadeaway of tissue edema surrounding the injury sits and ultimately improve the recovery of neural dysfunction [5].

Xuesaitong freeze-dry powder for injection used in this study is off-white to stramineous color that is powder in amorphous forms or loosened solid powder with bitter in taste and subtle sweet. It can relieve brain edema and inflammatory reaction so as to promote the absorption of intracranial hematoma [6]. Xuesaitong is a new type of thrombolysis anticoagulant, which has been widely used in clinic practice due to its beneficial effects on raising the cerebral blood flow, ameliorate the blood circulation of infarction zone and effectively prohibit the formation of thrombus. It exerts effects on brain protection under injured or anoxic condition through modifying blood circulation, taking against platelet aggregation and prolonging Clotting time [7].

Bax is an apoptosis promoted factor that commonly present in the cytoplasm as transducer in cellular damage and stimulation. However, when cellular apoptosis occurs, Bax that associates with mitochondrial may present in a manner of monomer without the activity. Or it may present in a manner of activated complex with big molecular weight that combined with mitochondria membrane [8] [9]. Responded to injury and stimulation, Bax will re-localize at surface of mitochondrial and further destroy the function of Bcl-2 protein that has anti-apoptotic effect in normal condition.

During the process of apoptosis, Bcl-2 gene family plays a critical role [10]. Bcl-2 gene family is divided into two kinds of protein-anti-apoptotic protein and promoting apoptotic protein. The product of Bax, an important factor attached to promoting apoptotic protein [11], has an effect on antagonize the bioactivity of bcl-2, which is homologous to Bax's product, an associated protein of bcl-2. Bax could form a bore spanning the mitochondrial outer membrane, resulting in reduction of the membrane potentials and outflow of Cytochrome C (Cyt C) and apoptosis inducing factor (AIF). Whereas Cyt C, combining with Apaf-1, ATP and pro-Caspase-9, form apoptosome compound, which activates Caspase-9. When binding to Cyt C, the combing capacity between Apaf-1 and pro-Caspase-9 was strengthened. Anti-apoptotic molecule Bcl-2 and Bcl-XL play a role in prohibiting the bore formation on the mitochondrial. When Bax, Bcl-2 and Bcl-XL form heterodimer, it blocks up the anti-apoptotic effects exerted by these molecules [7]. These anti-apoptotic effects may attributed to the underlying mechanism by destroying the mitochondrial outer membrane, promoting the assembly of death-inducing signing complex (DISC) and activity of Caspase-3, Caspase-8, ultimately playing roles in promoting apoptosis [11]. It has been demonstrated that expression of Bax closely associated with incidence and development of various malignant tumors [12] [13]. Moreover, it has been revealed that Bax could mediate the apoptotic sensibilization produced by fibroin of mammary gland. This provides new foundation for the treatment of breast cancer and prostatic cancer [14], suggesting Bax may play a crucial role in clinical tumor therapy. However, to our knowledge, the relationship between Bax and TBI is few reported and largely unknown.

Taken together, traumatic brain injury (TBI) model was employed in this study. NSS Score System was used to evaluate the therapeutic effect of Xuesaitong. Furthermore, qPCR was used for Bax expression detection. And the underlying action mechanism of Xuesaitong was investigated. Additionally, the relationship of Bax expression level and Xuesaitong's effects on TBI rats was elucidated.

2. Methods

2.1. Animal and Grouping

A total of 30 Sprague Dawley (SD) rats, weighing 200 - 240 g, provided by Experimental Animal Center of Kunming Medical University, were used in this study. The animal grouping was shown in **Table 1**. TBI group was TBI rats without Xuesaitong injection. Sham group was rats subjected to craniotomy and cerebral dura mater exposure only. Rats in TBI-Xuesaitong injection group underwent TBI + Xuesaitong injection. In TBI + Xuesaitong group, rats were fixed and Xusaitong solution (200 mg/bottle, Pharmaceutical Group co.ltd of Kunming, China) was injected at 8 am every morning via caudal vein daily from 1 to 14d after operation (200 mg/d).

2.2. Animal Model Preparation

The rat traumatic brain injury model was prepared by using weight free drop impact method [15] [16]. Briefly, SD rats were anesthetized intraperitoneally with 3.6% chloral hydrate (1 ml/100g). And then, rats were fixed in prone position. An incision was performed along the sagittal plane of the left parietal bone of skull, the left parietal bone was exposed. A 5.0 mm × 5.0 mm sized bone window was formed at approximately 2.5 mm aside from sagittal suture and 1.5 mm aside from coronal suture, the cerebral dura mater was then exposed. Subsequently, an iron midheaven cylinder, weighing 59 g, was allowed to drop from 10 cm height along with a metal pole. When it impacted on the clout placed on the surface of cerebral cortex, the contusion of left motor cortex formed, which could mimic the state of traumatic brain injury. Following the operation, the foods and water was *ad libitum*. Penicilline sodium (0.5 ml/20IU) was injected im. to prevent infection.

2.3. Neurological Severity Score Evaluation [17] [18]

Neurological Severity Scores (NSS) Rating Scale System was used to evaluate the motor function of rats following TBI. NSS assessment was conducted daily by blindness method from Day 1 to Day 14 after operation. The NSS Scales was shown in **Table 2**. SPSS software was employed to analyze the data.

The score ranged from 2 - 18. 13 - 18 score represented severe injury. 7 - 12 score represented moderate injury. While 1 - 6 score represented mild injury [19]. NSS Score also was used to evaluate whether or not the TBI rat models was installed successfully.

2.4. Real-Time RT-PCR

The brain tissues of rats were harvested from rats in all experimental groups on 1d post of TBI. Total RNA was isolated from brain tissues using Trizol reagent (Invitrogen) to produce cDNA, respectively. Then, quantitative RT-PCR (qRT-PCR) analysis was performed to analyze the level of Bax. QRT-PCR of cDNA was performed, and then the PCR reaction was performed at 95˚C for 2 min; and circulated 40 times at 95˚C for 20s, 50˚C for 30s and 60˚C for 40s. The fluorescence were collected and recorded after finishing 40 cycles. And the reaction was performed on real time fluorescent quantitative PCR (Thermal Cycler). Data were analyzed using a relative critical threshold (Ct) method where the amount of target normalized to the amount of inner control.

2.5. Statistics

Experimental data were expressed as mean ± standard error. *T* test was used for comparison samples between different two groups. Muti-sample comparison in the same group was analyzed by ANOVA. The SPSS 18.0

Table 1. Animal and grouping.

Groups	NSS/qPCR
TBI	10
Sham	10
TBI-Xuesaitong injection (via caudal vein)	10
Total	30

Table 2. Neurological Severity Scores (NSS).

Motors tests	Points
Raising rat by the tail	3
1 Flexion of forelimb	
1 Flexion of hindlimb	
1 Head moved >10˚ to vertical axis within 30s	
Placing rat on the floor (normal = 0; maximum = 3)	3
0 Normal walk	
1 Inability to walk straight	
2 Circling toward the paretic side	
3 Fall down to the paretic side	
Sensory tests	2
1 Placing test (visual and tactile test)	
2 Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	
Beam balance test (normal = 0; maximum = 6)	6
0 Balances with steady posture	
1 Grasps side of beam	
2 Hugs the beam and one limb falls down from the beam	
3 Hugs the beam and two limbs fall down from the beam, or spins on beam (>60s)	
4 Attempts to balance on the beam but falls off (>40s)	
5 Attempts to balance on the beam but falls off (>20s)	
6 Falls off: No attempt to balance or hang on to the beam (<20s)	
Reflexes absent and abnormal movements	4
1 Pinna reflex (head shake when touching the auditory meatus)	
1 Corneal reflex (eye blink when lightly touching the cornea with cotton)	
1 Startle reflex (motor response to a brief noise from snapping a clipboard paper)	
1 Seizures, myoclonus, myodystony	
Maximum points	18

Note: One point is awarded for the inability to perform the tasks or for the lack of a tested reflex; 13 to 18 indicates severe injury; 7 to 12, moderate injury; 1 to 6, mild injury.

software (IBM Company) was used for statistical analysis. A level of $P < 0.05$ was considered as statistical significant. When $P < 0.01$, it is considered as significant statistical difference.

3. Results

3.1. NSS Scores

The NSS Scores in TBI group and Xuesaitong treated group turned out to be a step-up trend compared with sham group. During 8 to 13 d after operation, there was significant difference in NSS scores within TBI group and Xuesaitong group respectively ($P < 0.01$). In these two groups, the NSS Scores had a tendency of rising over time after the operation up to 14 d. It is implied that with the time passed following injury, rats got some extent of natural recovery.

The NSS scores in TBI group was substantially higher than that of sham group ($P < 0.01$). There was no marked difference for NSS scored between Xuesaitong group and TBI group ($P > 0.05$). NSS Scales in different experimental groups were shown in **Table 3** and **Figure 1**.

3.2. qPCR

The level of Bax expression in TBI group was markedly higher than that of sham operative group and Xuesaitong treated group, respectively ($P < 0.01$). The comparison of Bax expression level among TBI, Xuesaitong treated and sham operative group was shown in **Table 4** and **Figure 2**.

Table 3. NSS Scales in different group.

DPO	NSS Scales (Mean ± standard error)		
	TBI group	Xuesaitong group	Sham group
1	5.2707 ± 0.61177	4.3328 ± 0.11655*	0.2462 ± 0.03078*
2	4.7017 ± 0.20472	4.6639 ± 0.11755*	0.0586 ± 0.00953*
3	4.3627 ± 0.11777	4.2630 ± 0.08558*	0.0000 ± 0.00000*
4	3.5687 ± 0.05369	3.5644 ± 0.05255*	0.0000 ± 0.00000*
6	2.3539 ± 0.08526	2.1195 ± 0.07089*	0.0000 ± 0.00000*
8	1.1968 ± 0.03799	0.8860 ± 0.03101*	0.0000 ± 0.00000*
10	1.0003 ± 0.03232	0.8410 ± 0.04815*	0.0000 ± 0.00000*
12	0.5975 ± 0.01911	0.5169 ± 0.01374*	0.0000 ± 0.00000*
14	0.2272 ± 0.02295	0.0949 ± 0.00402*	0.0000 ± 0.00000*

Note: *Represented compared with TBI group. When $P < 0.01$, there was significant difference. DPO represents days post of the operation.

Table 4. BAX relative optical density scales.

Group	BAX
TBI	0.0229 ± 0.0024
TBI+XueSaiTong	0.0175 ± 0.001*
Sham	0.0115 ± 0.002*

Note: *Represented compared with TBI group, when $P < 0.01$, there was significant difference.

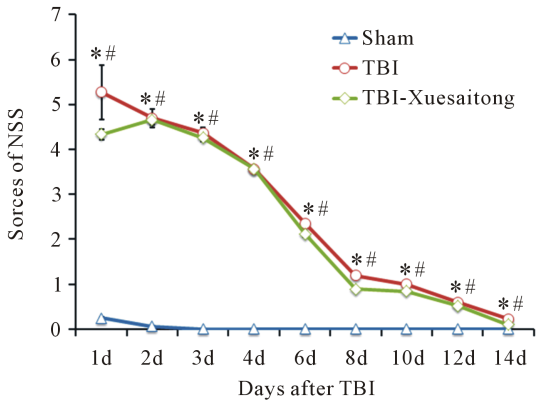

Figure 1. NSS Score polygram. *Represented comparison of inner groups in TBI and sham group. When $P < 0.05$ there was significant difference; #Represented inter-groups comparison between TBI and Xuesaitong group. When $P < 0.05$, it was considered statistically significant.

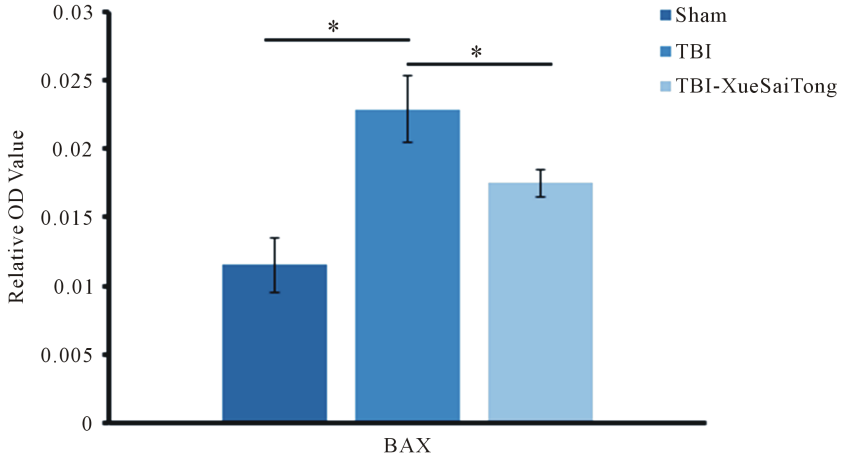

Figure 2. BAX relative optical density histogram. *Represented there was significant difference ($P < 0.01$) between the two group connected with the transverse lines.

4. Discussion

Traumatic brain injury is one of the most common diseases in neurosurgical clinics with high incidence rate and mortality [1] [2]. In this study, NSS Score Evaluation revealed that there was marked difference between TBI and sham operative group, suggesting that the TBI rat models were successfully installed. While there was nearly not any significant difference between any two subgroups on the time spots from 8 d to 13 d after the operation in TBI group, it was implied that TBI rats had some certain ability to recover spontaneously. However, as there was obvious difference in NSS Scores between TBI and sham operative group, it was indicative that the injury resulted from TBI could not recover completely, that is, the rats of TBI had functional defect to a certain extent. The third, our results in NSS Score evaluation found that there existed a step-up trend in NSS Scores in TBI and Xuesaitong treated group when compared with sham group respectively, and the NSS scores in Xuesaitong treated group had no marked difference between those of TBI group, suggesting that Xuesaitong did not exhibited active role in the Neural functional recovery in TBI rats in this study.

Following brain injury, there was local ischemia occurred in the tissues surrounding the injury site. The local ischemia would further lead to local tissue necrosis, *i.e.* cellular apoptosis [3]. Real-time PCR showed that the level of Bax expression in TBI group was markedly considerably higher than that of sham operative group. It was strongly suggested that brain injury did led to the augmentation of Bax gene expression, which thereby resulted in the apoptosis of nerve cells. Previous studies found that Bcl-2/Bax ratio had a close association with cellular apoptosis. Notably, when Bax and Bcl-2 formed homo/heterodimer, it could play a crucial role in regulating cellular apoptosis. When Bax formed homodimer, it could result in cellular apoptosis, while when bcl-2 formed heterodimer, it could attenuate apoptotic rate of nerve cells by markedly suppress Bax, ultimately play a vital role in brain tissue protection [19]. It is concluded from above that when the content of heterodimer (Bcl-XL/Bax and Bcl2/Bax) above 50% intracellularly, the cells commonly endure the apoptosis. Whereas when the content of Bax homodimer above 80% intracellularly, cellular apoptosis occurred [20]. Our results showed that the level of Bas expression in Xuesaitong treated group was markedly lower than that of TBI group. This demonstrated that Xuesaitong played roles in prohibiting Bax gene expression.

In summary, despite Xuesaitong could play its roles in brain tissue protection in brain injury as described in previous report [21], we found that it did not had any active and positive role in neural functional recovery when injected through caudal vein in TBI rats. Previous reported concluded that Xuesaitong may played its roles through suppressing Bax gene expression in that it not only could relieve the extent of brain edema and infarction area [22] [23], but also further ameliorate neural function, thereby ultimately contributing to the therapeutic effect of brain injury. However, currently, Xuesaitong was mostly used in the cerebral ischemia of rat models, even in clinical treatment, such as cerebral infarction therapy. Therefore, its concrete roles and associated underlying mechanism in brain injury await intensive studies. And the dose, pathway and timing for Xuesaitong administration are another important factors affecting its roles in brain protection. Furthermore, the development of brain injury may be involved in muti-mechanisms rather than a simple one. In this study, Bax gene expression

was detected only on 1 d post of operation. Because of at this time, NSS Scores change among the three experimental groups was the biggest; we wanted to investigate the relationship of NSS Score-changes and Bas gene expression. Notably, the scope of timing for Bax detection should be enlarged in future studies so that more convincing outcomes can be obtained. Therefore, our study is a preliminary one. It is needed to intensively elucidate the concrete actions taken by Xuesaitong in therapy of brain injury. Importantly, more reliable evidence involved in changes of Bax gene expression and Xuesaitong's roles in brain injury awaits professional study.

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References

[1] Zhang, G. and Zhi, K.D. (2009) Mechanism of Cognitive Impairment Following Traumatic Brain Injury. *Chin J Neuromed*, **8**, 637-639.

[2] Hao, S.-Y. and Liu, B.Y. (2005) Research Progress of Local Cerebral Ischemia Following Traumatic Brain Injury. *Foreign Medical Sciences Section on Neurology & Neurosurgery*, **32**, 430-434.

[3] Gan, Y., Xu, H.B. and Sun, X.B. (2007) Research Progress of Pharmacologic Actions of Panax Notoginsenosides. *Li Shi Zhen Medicine and Materia Medical Research*, **18**, 1251-1252.

[4] Li, Z. and Cui, R.-Q. (2013) Research Progress on Roles of Injection of Chinese Drugs in the Therapy of Cardiovascular and Cerebrovascular Diseases. *Traditional Chinese Medicine and Pharmacy of Nei Meng Gu*, **32**, 127-128.

[5] Zhao, X.-S. (2014) Experimental Study in Neuroprotective Roles of Panax Notoginsenosides in Rats with Cerebral Hemorrhage. Beijing Traditional Chinese Medicine and Pharmacy University, Beijing.

[6] Wang, Z.-J. (2013) Clinical Therapeutic Observation of 88 Cases Patients with Cerebral Thrombosi. *Medical Research of China and Overseas*, **11**, 166.

[7] http://www.ebiotrade.com/emgzf/Merck200403/Untitled-2.htm

[8] Pecina-Slaus, N. (2009) Genetic and Molecular Insights into Apoptosis. *Acta Medica Croatica*, **2**, 13-19.

[9] Kastelan, M., Massari, L.P. and Brajac, I. (2010) The Role of bcl-2 Family Proteins in Psoriasis. *Lijec Vjesn*, **132**, 31-33.

[10] Tang, X.Y. and Pu, X.Y. (2011) Cloning and Expression of Mouse Bax Gene. *Lab Med Clin*, **8**, 662-664.

[11] Ionoc, Y., Yamamoto, H., Krajewski, S., Reed, J.C. and Perucho, M. (2000) Mutational Inactivation of the Proapoptotic Gene BAX Confers Selective Advantage during Tumor Clonal Evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 10872-10877. http://dx.doi.org/10.1073/pnas.190210897

[12] Krajewska, M., Fenoglio-Preiser, C.M., Krajewski, S., Song, K., Macdonald, J.S., Stemmerman, G. and Reed, J.C. (1996) Immunohistochemical Analysis of Bcl-2 Family Proteins in Adenocarcrinomas of the Stomach. *The American Journal of Pathology*, **149**, 1449-1457.

[13] Liu, J., Yin, S., Reddy, N., Spencer, C. and Sheng, S.J. (2004) Bax Mediates the Apoptosis-Sensitizing Effect of Maspin. *Cancer Research*, **64**, 1703-1711. http://dx.doi.org/10.1158/0008-5472.CAN-03-2568

[14] Finnie, J.W. and Blumbergs, P.C. (2002) Traumatic Brain Injury. *Veterinary Pathology*, **39**, 679-689. http://dx.doi.org/10.1354/vp.39-6-679

[15] Huo, Y.-Q. and Tang, Y.-F. (2007) A Kind of Brain Traumatic Model of Modified Following Weight. *Journal of Guangxi Medical University*, **24**, 217-219.

[16] Chen, J.L., Li, Y., Wang, L. and Zhang, Z.G. (2001) Therapeutic Benefit of Intravenous Administration of Bone Marrow Stromal Cells after Cerebral Ischemia in Rats. *Stroke*, **32**, 1005-1011. http://dx.doi.org/10.1161/01.STR.32.4.1005

[17] Germano, A.F., Dixon, C.E., d'Avella, D., Hayes, R.L. and Tomasello, F. (1994) Behavioral Deficits Following Experimental Subarachnoid Hemorrhage in the Rat. *Journal of Neurotrauma*, **11**, 345-353. http://dx.doi.org/10.1089/neu.1994.11.345

[18] He, P., Zhu, D.-L., Zhu, P., Wang, R.-J. and Wang, X. (2012) The Effect of Total Panax Notoginseng Naponinsin on the Apoptosis of Brain Cells and the Expression of Apoptotic Protein Bax in Newbron Rat after Asphyxia. *Journal of Clinical Pediatrics*, **30**, 1178-1181.

[19] Wang, W.D. and Chen, Z.T. (2007) Bel-2/Bax Ratio and the "Life or Death Fate" of Cells. *Chinese Journal of Cancer Biotherapy*, **14**, 393-396.

[20] Chen, Y., Constantini, S., Trembovler, V., Weinstock, M. and Shohami, E. (1996) An Experimental Model of Closed Head Injury in Mice: Pathophysiology, Histopathology, and Cognitive Deficits. *Journal of Neurotrauma*, **13**, 557-568.

[21] Zhang, X.M., Wu, J.R. and Zhang, B. (2015) Xuesaitong Injection as One Adjuvant Treatment of Acute Cerebral Infarction: A Systematic Review and Meta-Analysis. *BMC Complementary and Alternative Medicine*, **15**, 36. http://dx.doi.org/10.1186/s12906-015-0560-4

[22] Xi, W.-G. and Zhao, Z.-Y. (2009) Roles of PNS in Rat Brain Ischemia of Thrombogenicity. *Chinese Herbs*, **40**, 234-236.

[23] Qiao, C.-L., Ding, Y.-F. and Yang, C.-R. (2012) Pharmacologic Research Progress of Notoginseng Total Saponins. *Modern Chinese Medicine*, **14**, 25-30.

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Not All "BAD" Cholesterol Carriers Are Necessarily Bad and Not All "GOOD" Cholesterol Carriers Are as Good as Can Be: Plasma Delipidation, a Non-Pharmacological Treatment for Atherosclerosis

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Abstract

More than four decades ago it was established that an elevated low-density lipoprotein-cholesterol level was a risk for developing coronary artery disease. For the last two decades, statins have been the cornerstone of reducing low-density lipoprotein-cholesterol, but despite significant clinical efficacy in the majority of patients, a large number of patients suffer from side effects and cannot tolerate the required statin dose to reach their recommended low-density lipoprotein-cholesterol goals. Preliminary clinical studies indicate that monoclonal antibodies to PCSK9 appear to be highly efficacious in lowering low-density lipoprotein-cholesterol with a favourable adverse event profile. However, further longer-term clinical studies are required to determine their safety. From the early-proposed concept for high-density lipoprotein-mediated cholesterol efflux for the treatment of coronary artery disease, the concentration of the cholesterol content in high-density lipoprotein particles has been considered a surrogate measurement for the efficacy of the reverse cholesterol transport process. However, unlike the beneficial effects of the statins and monoclonal antibodies to PCSK9 in reducing low-density lipoprotein-cholesterol, no significant advances have been made to increase the levels of high-density lipoprotein-cholesterol. Here it is shown that by a non-pharmacological plasma delipidation means, the atherogenic low-density lipoproteins can be converted to anti-atherogenic particles and that the high-density lipoproteins are converted to particles with extreme high affinity to cause rapid regression of atherosclerosis.

Keywords

Plasma Delipidation, Lipid Apheresis, Regression, Atherosclerosis, Pre-β HDL

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1. Introduction

1.1. Early Observations of Lipoproteins and Their Relationships with Atherosclerosis

More than 40 years ago, the Framingham Heart Study established that an elevated low-density lipoprotein-cholesterol (LDL-C) level was a risk for developing coronary artery disease (CAD) [1].

Also, more than 40 years ago, Glomset [2] proposed that high-density lipoprotein (HDL) transfers cholesterol from peripheral tissues to the liver, where metabolites of the sterol are excreted into the bile. From the early proposed concept for HDL-mediated cholesterol efflux [3], the concentration of the cholesterol content in HDL particles has been considered a surrogate measurement for the efficiency of the reverse cholesterol transport (RCT) process.

In the 1970s it was well established that plasma contained lipid carriers, some of which were detrimental to the body, LDLs, and others that were beneficial to the body, HDLs.

1.2. Atherosclerosis Formation

Lipoproteins carry cholesterol to and from the vessel wall; if too much cholesterol is carried to and too little is carried from the vessel, it accumulates there and causes fatty deposits to build up in the vessel wall, a phenomenon known as atherosclerosis.

Atherosclerosis, the horror of mankind, refers to the build-up of cholesterol, other fats, and other substances such as calcium deposits, cells, cell debris in, and on, artery walls (plaques), which can restrict blood flow.

Blood clots can form at the plaque and either obstruct blood flow, or, break off and clog the arteries such as the coronary arteries to the heart or carotid arteries to the brain, resulting in a possible heart attack or stroke respectively.

During the pathogenesis of atherosclerosis, blood monocytes, originated from bone marrow-derived progenitor cells, infiltrate from blood to the intima and sub intima, a process that is activated by subendothelial accumulation of apolipoprotein B-containing lipoproteins (LDLs).

The accumulation of apolipoprotein B-lipoproteins (Apo B-LPs) in the matrix beneath the endothelial cell layer leads to the recruitment of monocytes. These cells differentiate into macrophages and undergo foam cell formation.

Macrophage scavenger receptor (SR) binds and internalise modified forms of LDL through mechanisms that are not inhibited by cellular cholesterol content and they are likely responsible for macrophage cholesterol accumulation.

1.3. Prevention of Atherosclerosis

Over the past decade, statins, with maintaining a healthy lifestyle, have been the most effective therapies in treating atherosclerosis by reducing the synthesis and thus delivery of cholesterol in Apo B-LPs to the vessel wall. This has resulted in decreased incidences of heart disease by 30 per cent.

There is, however, an urgent need for further reduction of the unacceptably high remaining risk of cardiovascular and cerebrovascular diseases.

Coronary heart disease (CHD) and stroke, caused by atherosclerosis, are leading causes of death in the USA. More than 80 million Americans, one in three of the population, suffer from atherosclerotic disease; most don't even know it. More than 2600 Americans die every day because of cardiovascular diseases, approximately 1 death every 33 seconds.

In essence, drugs like the statins can be regarded as preventative measures of atherosclerosis, that is, statins may inhibit the progression of the disease, which on its own is a major achievement. However, there are no convincing data to show that the statins can cause regression of atherosclerosis.

The monoclonal antibodies alirocumab and evolocumab that block the gene PCSK9 show promise to reduce LDL-C even more effectively than statins. The need to combat atherosclerosis is recognized worldwide. Consequently, health authorities are being forced to approve these promising new drugs years before completion of the necessary clinical trials. Definitive evidence of the drugs' safety and effectiveness in reducing heart attacks and deaths will only be obtained after such trials are completed.

1.4. Atherosclerosis Regression

To date, the best plausible mechanism to achieve regression of atherosclerosis is by RCT.

In a nutshell, cholesterol, synthesised or deposited in peripheral tissues is returned to the liver by RCT in which HDL plays a central role. HDL is secreted by the liver and intestine in the form of nascent particles consisting of phospholipid and apolipoprotein A1 (Apo A1). Nascent HDL interacts with peripheral cells, such as macrophages, to facilitate the removal of excess free cholesterol (FC), a process facilitated by the ATP-binding cassette protein 1 (ABCA1). FC is generated in part by the hydrolysis of cellular cholesteryl ester (CE) stores by the enzyme cholesterol ester hydrolase (CEH). FC in HDL is then reconverted into CE resulting in mature CE-rich HDL as a result of the plasma cholesterol-esterifying enzyme lecithin: cholesterol acyltransferase (LCAT). Selective uptake of the HDL by the liver is mediated by the scavenger receptor class-B, type I (SR-BI). CE in the liver derived from HDL contributes to the hepatic-cholesterol pool by processes involving CEH and is used for bile acid synthesis. Cholesterol is eventually excreted from the body or reabsorbed either as its metabolites bile acids or as FC in the bile.

Another, not very publicized, pathway for RCT involves the widely accepted atherogenic lipoproteins and the plasma enzyme CE transfer protein (CETP). CE can be transferred from HDL to Apo B-LPs, such as VLDLs and LDLs, by CETP. Through uptake of LDL by the liver via hepatic LDL receptors, cholesterol can then be transported to the liver, where it contributes to the hepatic-cholesterol pool for excretion or reabsorption.

1.5. Regression of Atherosclerosis by Stimulation of RCT

Approaches to obtain regression of atherosclerosis are avidly being investigated. One such approach is the movement of cholesterol from within the body to outside of the body by non-pharmacological methods.

2. Methods

Plasma Delipidation

As early as in the 1970s it was proposed that removing lipid from the plasma by non-pharmacological means, but retaining their protein carriers, the apolipoproteins, in the delipidated plasma may have application to treat hyperlipidemic diseases [4] [5] and in 1978 the importance of apolipoproteins in lipid metabolism was highlighted [6].

By studying and understanding lipid-protein interactions and the interconnectivity of organic solvents with the binding of lipid to proteins, it was considered useful to develop a plasma delipidation process that would result in:

- removal of lipid from plasma without protein denaturation;
- simultaneous extraction of different classes of lipids but at different rates;
- extraction of partial or total lipids from different lipoprotein classes but at different rates.

The rationale of the plasma delipidation system has previously been described [7].

3. Results

3.1. Basic Research

In 1976 it was first reported that a mixture of butanol and diisopropyl ether (DIPE) attained removal of triglycerides, FC, EC, phospholipids (except gangliosides [7] [8]), and unesterified fatty acids from plasma without protein denaturation. Different lipids were simultaneously extracted, but the rate of extraction was most rapid for unesterified fatty acids, followed by triglycerides, CE, FC and phospholipids at, respectively, decreasing rates [4] [5].

Delipidation of plasma yielded ordered delipidation of HDL, very low-density lipoprotein (VLDL) and LDL in plasma. HDL was more readily delipidated than were LDL and VLDL fractions [4] [5]. Only lipids were removed from the delipidated plasma, all other constituents of plasma including the apolipoproteins remained intact, to the extent that delipidated plasma is used to measure apolipoproteins concentrations [7] [9]. The concentrations and activities of enzymes, including lipid-associated enzymes, were unaffected by the delipidation process [4] [7] [9]-[11].

3.2. Preclinical

Cell Culture Studies

Cellular binding activity of LDL and cholesterol-free LDL, obtained by delipidation of plasma with DIPE- butanol, are identical [12]. Delipidated LDL does not stimulate cellular cholesterol ester (ACAT) synthesis, indicating that no cholesterol is presented to those cells by delipidated LDL [12]. Delipidation of plasma converts the spherical HDL particles to disc and amorphous forms but this does not alter receptor-binding activity [12]-[14].

Delipidated serum, resulting in lipid depletion of all lipoprotein classes, effluxes cholesterol from human mononuclear leukocytes. When delipidated serum substantially depletes the contents of cellular cholesterol, stimulation of intracellular sterol synthesis occurs by a factor of eight to eventually meet the needs of cholesterol for the survival of the cells [15].

However, intracellular sterol synthesis is inhibited by the addition of non-delipidated LDL to the delipidated serum. This indicates that cells preferentially utilize cholesterol given to them rather than synthesizing their own [15]. These studies were confirmed using skin and lung fibroblasts. The activity of HMG-CoA reductase (the rate limiting enzyme for the synthesis of cholesterol) in these cells, increased 5 to 30 fold in media containing delipidated serum. This occurred only after substantial removal of endogenous cellular cholesterol resulting in cellular cholesterol depletion, caused by the delipidated serum [13]. Addition of non-delipidated LDL to the cells resulted in the inhibition of cholesterol synthesis within the cells [14].

It was subsequently established that the component in the delipidated serum responsible for the removal of cholesterol from cells grown in culture was delipidated HDL and that delipidated HDL was a much better (more than 6 fold) cholesterol acceptor than non-delipidated HDL [14].

Thus, delipidated serum contained delipidated LDL that bound to LDL cell receptors but no cholesterol was supplied to these cells. Delipidated serum also contained delipidated HDL that interacted with HDL cell receptors, in this case the modified HDL was capable of removing much more cholesterol from cells compared with HDL particles in non-delipidated serum. It was concluded that delipidated serum supplied less cholesterol to cells and removed more cholesterol from cells.

3.3. Clinical

3.3.1. Batch Type Lipid Apheresis

A plasma delipidation therapy evolved and was termed lipid apheresis (LA). Initially, batch type extracorporeal lipid apheresis was applied to animals. Twenty five percent of plasma was removed from normolipidemic and diet-induced hyperlipidemic roosters. The plasmas were delipidated and autologously returned back to the same animals.

Different responses were observed when hyperlipidemic animals were compared with normolipidemic animals. In hypercholesterolemic LA-treated animals, an excessive cholesterol pool was rapidly mobilized when compared to normocholesterolemic animals [16].

In the normolipidemic animals the reduction of total cholesterol in plasma was immediate and was significantly sustained for up to 150 minutes. Sixteen hours after LA, the concentrations of total cholesterol in plasma had returned to pre-treatment values. In contrast, with the hypercholesterolemic animals, LA reduced the plasma level of total cholesterol only very transiently. Fifteen minutes after LA, total cholesterol was significantly reduced in the plasma of the hypercholesterolemic animals. Thirty minutes and thereafter, following LA treatment, the concentration of total cholesterol in plasma was generally lower than the pre-treatment value but was not statistically different from the pre-treatment value. Haematological and biochemical parameters in the blood were unaffected by LA treatment [16].

These data strongly suggested that more of an excessive and accessible cholesterol pool in the hypercholesterolemic animals existed that was rapidly mobilizable to the plasma compartment by the LA treatment and supported the cell culture studies.

Virtually immediately after infusion of delipidated plasma, conspicuous changes occurred in HDL. LA markedly induced pre-β HDL and at least three other “new” HDL particles in the vascular system and these changes were transient. At approximately 150 minutes after LA, the changes in the HDL pattern had almost returned to the original pre-LA treatment patterns. Concomitant to the changes in the HDL pattern was the immediate reduction in plasma cholesterol concentrations, which was sustained also for 150 minutes [17].

Saturated LCAT kinetics was observed for 150 minutes after reinfusion of delipidated plasma in the hypercholesterolemic animals but not normocholesterolemic animals, suggesting that a larger pool of FC was being mobilized in the hypercholesterolemic animals [18].

Cholesterol ester transfer activity (CETA) was twice as high in delipidated hyperlipidemic plasma than in delipidated normolipidemic plasma [19]. This, together with the LCAT kinetic observations, indicated that in the hyperlipidemic state, more CE was being transferred from relipidated HDL to delipidated LDL.

Batch LA-treatments on five consecutive days corresponding to treatments of 1.25 of body plasma volume, resulted in markedly reduced lipid in the arterial wall as well as marked increases in the lumen diameter [20].

Substantive mesenterical adipose tissue was clearly present in the abdomen of diet and hormone induced hyperlipidemic animals. This pool of lipid was drastically diminished when the hyperlipidemic animals were treated by two LA procedures corresponding to LA of 0.5 body plasma volume [21].

3.2.2. Continuous Lipid Apheresis

Subsequent to the batch type LA procedure, a continuous LA process was developed in early 1990 [22].

Application of the continuous LA procedure to bovine [23] and porcine [24] normocholesterolemic and diet-induced hypercholesterolemic animals revealed that LA of 1 to 3.5 plasma volumes over 4 to 6 occasions resulted in the following:

- Removal of lipids from plasma by the organic solvent extraction
- Haematological and biochemical parameters were unchanged
- Post-mortem studies showed no adverse effects
- During LA treatment pre-beta and other “new” HDL particles were transiently present in plasma
- LDL was also delipidated
- Rapid, but transient reductions in plasma cholesterol occurred as a consequence of each LA treatment
- After LA much less time was required with the hypercholesterolemic animals for the plasma cholesterol concentration to return to pre-treatment values when compared to normocholesterolemic animals
- Saturated LCAT kinetics occurred in LA treated hypercholesterolemic animals but not normocholesterolemic animals
- CETA was higher in LA treated hyperlipidemic animals than in normolipidemic animals
- Apolipoproteins contents were unaffected by LA treatment
- LA in hyperlipidemic animals mobilized pre-administered radio labelled cholesterol
- Hepatic HMG-CoA reductase activities in the hypercholesterolemic animals were reduced when compared with normocholesterolemic animals
- LA treatment of hypercholesterolemic animals did not result in increases of HMG-CoA reductase activity or plasma lathosterol concentrations
- Histological studies on various segments of arteries indicated removal of lipid from arteries and improvement in morphology of arteries as a consequence of LA treatment

It was interesting that, with these three distinctly different animal models, LA resulted in similar observations.

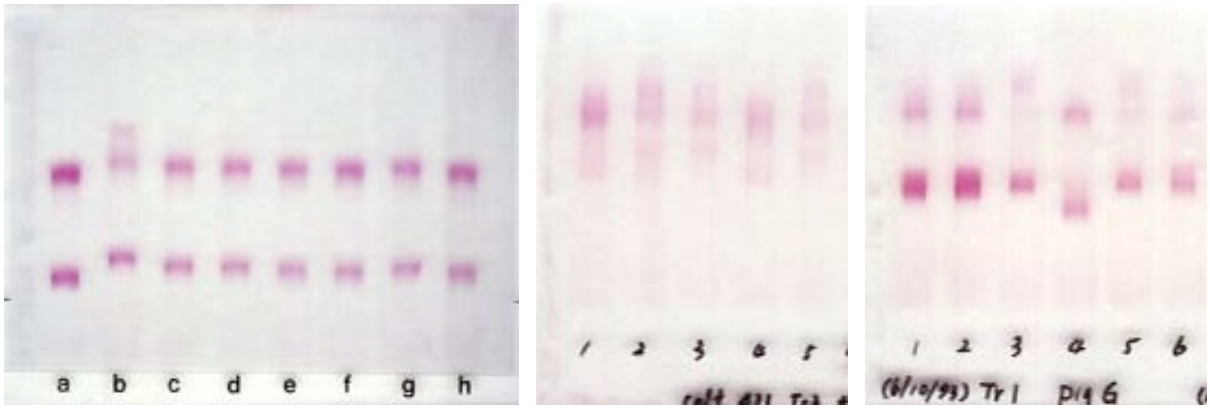
Figures 1(a)-(c) show the changes in electropherograms, before (A, a; B, 1; C, 1), during, and after LA (A, b-h; B, 2-5; C, 2-6) in the three different models (rooster, bovine and porcine). Obvious, but transient changes in the HDL region were observed. The lipoproteins were stained for lipid. All of the observed “new” HDLs as a consequence of LA, reacted with Apo A1 antibodies.

The LA treatment of the hypercholesterolemic roosters resulted in remarkable reduction of the intensity of lipid in the arterial wall as well as marked increases in lumen diameter.

Figure 2 shows a representative section of a lesion with significant narrowing of the lumen of the thoracic aorta of a hypercholesterolemic rooster and a representative section of a similar site of the thoracic aorta of a hypercholesterolemic animal that had undergone five LA treatments.

Higher magnification of the media sections of the abdominal aorta in hypercholesterolemic roosters showed the presence of intimal accumulation of lipid, whereas, LA treatment of hypercholesterolemic roosters had little such lipid accumulation (**Figure 3**).

Similar, but less pronounced observations were apparent when hypercholesterolemic bovine and porcine animals were subjected to LA. Histological studies revealed that LA treatments of these hypercholesterolemic animals resulted in removal of lipid from the various arteries studied.



(a) (b) (c)

Figure 1. Representative agarose electropherograms of plasma before and various times after introduction of delipidated autologous plasma into the vascular pool in hypercholesterolemic roosters (a), calves (b) and pigs (c). Lipids in the lipoprotein fractions were stained with Fat Red 7B. Multiple forms of HDLs that reacted to Apo A1 antibodies were observed soon after autologous delipidated plasma was administered intravenously into the animals. These observed changes were transient.

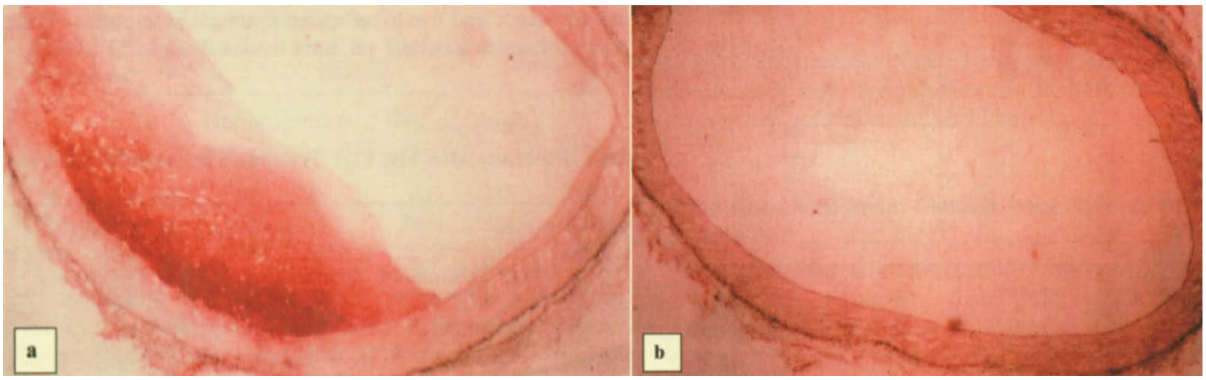


Figure 2. Histological sections (magnified x50) of thoracic aortas stained with Oil Red O. (a) Aorta from hypercholesterolemic rooster showing lipid-rich atherosclerotic plaque and significant luminal narrowing; (b) Aorta from hypercholesterolemic rooster after 5 LA treatments showing no evidence of atherosclerosis.

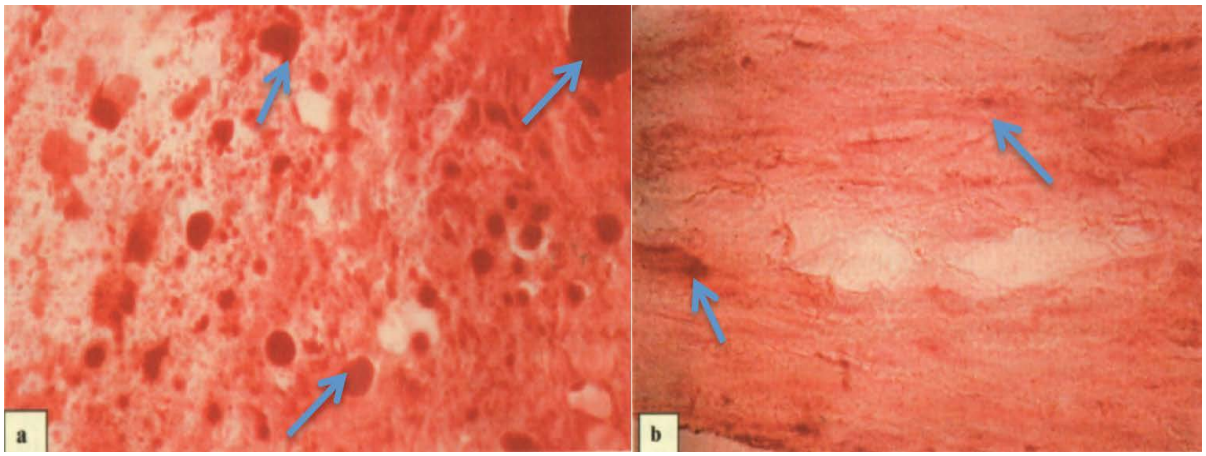


Figure 3. Histological sections (magnified ×800) of abdominal aortas stained with Oil Red O. (a) Aorta from hypercholesterolemic rooster showing extensive intimal accumulation of lipid (see arrows); (b) Aorta from hypercholesterolemic rooster after 5 LA treatments showing little lipid accumulation (see arrows).

3.3.3. Selective HDL Lipid Apheresis

Based on the observations reported in the 1970s, that plasma could be delipidated with butanol-ether to yield selective delipidation of HDL; a study was undertaken in non-human primates in the mid 2000s. Twelve weekly LA treatments, corresponding to a total infusion of approximately 4 body plasma volumes of selective HDL delipidated plasma of diet-induced hyperlipidemic African Green Monkeys gave similar results to those obtained with the avian, bovine and porcine models. Again it was shown that LA converted cholesterol-rich HDL particles to cholesterol-poor small, and pre-β-like HDLs. Pre-β HDL had a plasma residence time of 8 ± 6 h and was converted entirely to large αHDL, this was within the range of the residence time of pre-β HDL observed in the LA treated avian, bovine and porcine models. LA treatment with delipidated plasma reduced diet-induced aortic atherosclerosis in monkeys measured by intravascular ultrasound (IVUS) [25].

In all the animal studies, no serious adverse effects were observed with LA treatments. The promising observations of safety and efficacy achieved by LA treatment with the animal studies led to a randomized, placebo-controlled study to evaluate the safety and feasibility of autologous selective delipidated HDL plasma infusions in human patients with acute coronary syndrome (ACS) [26].

Seven weekly LA treatments of plasma with butanol-ether resulting in selective HDL delipidation, corresponding to a total infusion of approximately two body plasma volumes of selective HDL delipidated plasma, over seven weeks treatment in man, have shown to be feasible and well tolerated in patients with ACS. The levels of the preß-like HDL and αHDL in the delipidated plasma transiently converted from 5.6% to 79.1% and 92.8% to 20.9%, respectively, and confirmed the observations of LA treatment with the avian, bovine, porcine and monkey models. Patients underwent IVUS evaluation of the target vessel during the catheterization of ACS and up to 14 days following the final LA session. The IVUS data demonstrated a numeric trend toward regression in the total atheroma volume of −12.18 ± 36.75 mm^3 in the delipidated group versus an increase of total atheroma volume of 2.80 ± 21.25 mm^3 in the control group ($p = 0.268$, N.S.) [26].

The regressions of atherosclerosis although not significant in the humans and monkeys, were similar to the results reported in Apo A1 Milano studies [27].

On the contrary, the regression of atherosclerosis induced by LA in the rooster, bovine and porcine models were significant. These observed differences may be explained by the different precise conditions of the delipidation processes used with the various studies.

With the human and monkey models, the main focus was selective delipidation of HDL in plasma. Whereas, with the rooster, bovine and porcine models, all the lipoprotein classes in the plasma were delipidated. Efficacious mobilization of endogenous lipid stores including regression of atherosclerosis were obtained when plasma delipidation resulted in the removal of over 90% of cholesterols and triglycerides but retaining over 50% of phospholipids [7] [9] [16]-[18] [20]-[22]. Regression was also related to the delipidated plasma volume and frequency of LA treatment.

4. Discussion

4.1. Homeostasis versus Non-Homeostasis

The current accepted concept of increased LDL-C levels being a risk for developing CAD and increased HDL-C levels contributing to the efficiency of RCT are both derived from systems that are at homeostasis in which metabolic equilibria have been achieved.

Lipoproteins carry cholesterol to and from the vessel wall; if too much cholesterol is carried to and too little is carried from the vessel, it accumulates there and causes fatty deposits to build up in the vessel wall.

Conversely, if less cholesterol is carried to and more is removed from the vessel, it depletes fatty deposits from the vessel wall and this is achieved with LA.

During the application of LA, homeostasis is interrupted and a new transient dynamic equilibrium takes place.

Understanding the difference between homeostasis and dynamic transient equilibrium is essential to appreciate how LA results in regression of atherosclerosis.

Relatively high HDL-C plasma levels do not necessarily reflect the efficacy and anti-atherogenecity of RCT. The efficiency of cholesterol flux through RCT pathways is clinically more relevant than the HDL-C concentrations. The concentrations of HDL subclasses, the mobilization of cellular lipids for efflux by delipidated HDL and LDL, and the kinetics of relipidated HDL and LDL metabolism are important determinants of RCT and the risk of atherosclerosis. These dynamic transient equilibrium sequelae regarding the positive effects of stimula-

tion of CETP to increase RCT are contrary to the understanding of the inhibition concept of CETP to increase HDL-C as is the case with homeostasis.

4.2. Lipid Apheresis Shows Convincing Promise as a Treatment for Atherosclerosis

The experimental data of cell culture and clinical studies strongly indicate cholesterol efflux caused by LA is not related to the novo synthesis of cholesterol but is related to mobilization of existing cholesterol stores.

4.3. Mechanism of Action of Lipid Apheresis Resulting in Regression of Atherosclerosis

A proposed mechanism of action is summarized in **Figure 4** and is based on preclinical and clinical observations.

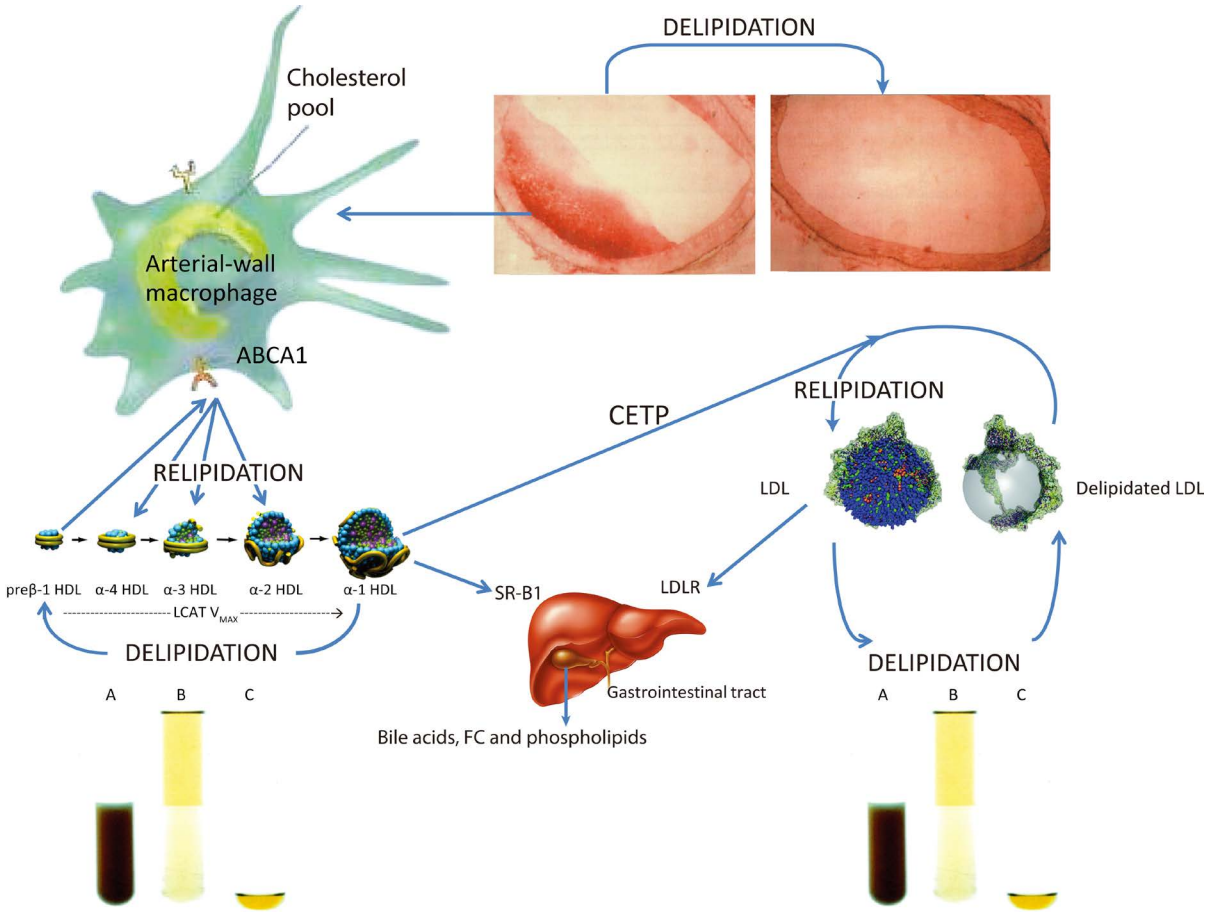


Figure 4. Proposed mechanism of action of LA-induced RCT. Delipidation by LA removes lipid from plasma (test tubes at, 5 and 8 o'clock) (A, undelipidated hyperlipidemic plasma; B, delipidated plasma, the upper organic solvent phase contains the lipids, the lower aqueous phase contains all plasma constituents but no lipids; C, recovered lipids extracted from the hyperlipidemic plasma). α-1 HDL is converted to preβ-1 HDL and when administered to the vascular pool, preβ-1 HDL interacts with ABCA1 receptors of arterial wall macrophages and binds with FC to produce, relipidated α-4 HDL, α-3 HDL and α-2 HDL (9 o'clock). During relipidation of HDL, LCAT activity is at Vmax converting FC to CE in the HDLs (9 o'clock). α-1 HDL is transported to the liver where it interacts with SR-B1 (6 o'clock) and its CE content is deposited in the liver. α-1 HDL can also transfer its CE to delipidated LDL with the help of stimulated CETP (3 o'clock). Relipidated LDL is transported to the liver and interacts with LDLR and its CE content is deposited in the liver (6 o'clock). The CE in the liver delivered by relipidated HDL and relipidated LDL is converted to FC by the enzyme CEH. FC is partly metabolized to bile acids and both FC and bile acids are secreted through the gastrointestinal tract to the intestine for reabsorption or excretion from the body. HDL = high-density lipoprotein; LDL = low-density lipoprotein; ABCA1 = adenosine triphosphate-binding cassette-A1; LCAT = lecithin cholesterol acyltransferase; CETP = cholesteryl ester transfer protein; LDLR = low-density lipoprotein receptor; SR-B1 = scavenger receptor B1.

The explanatory notes are indicated by a clock position using the analogy of a 12-hour clock. 5 and 8 o'clock show that plasma delipidation results in the extraction and removal of lipid from plasma (test tubes). As a consequence of plasma delipidation, α-1 HDL is converted to preß-1 HDL and when administered to the vascular pool interacts with ABCA1 receptors of arterial wall macrophages and binds with FC to produce relipidated α-4 HDL, α-3 HDL and α-2 HDL (9 o'clock). During this process CEH within the macrophage is activated and converts CE to FC, which binds to the delipidated HDL. Throughout these events LCAT activity works at maximum velocity converting FC to CE within the relipidated HDL in the plasma (9 o'clock). During these processes CE is removed from the macrophage, ultimately ending up in relipidated α-1 HDL.

α-1 HDL then has various choices, it can be transported in the vascular system to the liver where it interacts with SR-B1 receptors releasing its cholesterol content to the liver. Liver CEH converts CE to FC, which is partly converted to bile acids. FC and bile acids are secreted through the gastrointestinal tract to the intestine for reabsorption or excretion from the body.

Another choice of relipidated α-1 HDL is, with the help of CETP, to transfer its CE content to delipidated LDL (3 o'clock). In so doing, delipidated LDL, obtained by delipidation of plasma and administered into the vascular pool, is relipidated. In the course of these events CETP is activated. Relipidated LDL can then be transported to the liver, and interact with LDLR. Consequently the CE content of relipidated LDL may undergo the same fate as CE supplied to the liver by HDL, resulting in excretion of FC or its metabolites, bile acids.

These kinetic and dynamic effects of LA on the body are transient and occur during and temporarily after LA.

Consequently, LA results in reversal of atherosclerosis (12 o'clock).

5. Conclusions

RCT is an essential physiologic process that maintains peripheral and total body cholesterol homeostasis. The relationship between RCT and atherosclerosis has long been hypothesised. The molecular mechanisms regulating stimulation of macrophage RCT by plasma delipidation have now been largely elucidated.

The data presented in this report provide insights into the molecular regulation of RCT and indicate that the dynamic rate of macrophage RCT is more strongly associated with atherosclerosis than the steady-state plasma concentration of HDL-C.

Plasma delipidation therapy makes available essential key elements within the body that stimulate RCT resulting in regression of atherosclerosis.

Further work is continuing to establish the optimum delipidation conditions to obtain maximum regression of atherosclerosis, in a manner, that will have wide clinical applications in patients.

References

[1] Kannel, W.B., Castelli, W.P., Gordon, T. and McNamara, P.M. (1971) Serum Cholesterol, Lipoproteins, and the Risk of Coronary Heart Disease: The Framingham Study. *Annals of Internal Medicine*, **74**, 1-12. http://dx.doi.org/10.7326/0003-4819-74-1-1

[2] Glomset, J.A. and Wright, J.L. (1964) Some Properties of a Cholesterol Esterifying Enzyme in Human Plasma. *Biochimica et Biophysica Acta*, **89**, 266-276. http://dx.doi.org/10.1016/0926-6569(64)90215-9

[3] Glomset, J.A. (1968) The Plasma Lecithins: Cholesterol Acyltransferase Reaction. *Journal of Lipid Research*, **9**, 155-167.

[4] Cham, B.E. and Knowles, B.R. (1976) A Solvent System for Delipidation of Plasma or Serum without Protein Precipitation. *Journal of Lipid Research*, **17**, 176-181.

[5] Cham, B.E. and Knowles, B.R. (1976) Changes in Electrophoretic Mobilities of Alpha and Beta Lipoproteins as a Result of Plasma Delipidation. *Clinical Chemistry*, **22**, 305-309.

[6] Cham, B.E. and Knowles, B.R. (1976) *In Vitro* Partial Relipidation of Apolipoproteins in Plasma. *The Journal of Biological Chemistry*, **251**, 6367-6371.

[7] Cham, B.E. and Chase, T.R. (2013) Intravascular Infusion of Autologous Delipidated Plasma Induces Antiatherogenic Lipoproteins and Causes Regression of Atherosclerosis—Studies in Non-Primates, Monkeys and Humans. *Health* (*Special Issue on Atherosclerosis in Cardiovascular Disease*), **5**, 19-33.

[8] Wong, C.G. and Ladisch, S. (1983) Retention of Gangliosides in Serum Delipidated by Diisopropyl Ether-Butanol Extraction. *Journal of Lipid Research*, **24**, 666-669.

[9] Cham, B.E. (2007) Manipulation of Reverse Cholesterol Transport System—An Exploration for Rapid Regression of

Atherosclerosis. *Research Journal of Biological Sciences*, **2**, 291-300.

[10] Groener J.E.M., Van Rozen, A.E. and Erkelens, D.W. (1984) Cholesteryl Ester Transfer Activity. Localization and Role in the Distribution of Cholesteryl Ester among Lipoproteins in Man. *Atherosclerosis*, **50**, 261-271. http://dx.doi.org/10.1016/0021-9150(84)90074-1

[11] Groener, J.E.M., Pelton, R.W. and Kostner, G.M. (1986) Improved Estimation of Cholesteryl Ester Transfer/Exchange Activity in Serum or Plasma. *Clinical Chemistry*, **32**, 283-286.

[12] Innerarity, T.L. and Mahley, R.W. (1978) Enhanced Binding by Cultured Human Fibroblasts of Apo-E-Containing Lipoproteins as Compared with Low Density Lipoproteins. *Biochemistry*, **17**, 1440-1447. http://dx.doi.org/10.1021/bi00601a013

[13] Shakespeare, V. and Postle, A.D. (1979) Regulation of Cholesterol Synthesis in Skin Fibroblasts Derived from Old People. *Atherosclerosis*, **33**, 359-364. http://dx.doi.org/10.1016/0021-9150(79)90187-4

[14] Slater, H.R., Smith, E.B. and Roberston, F.W. (1980) The Effect of Delipidated High-Density Lipoprotein on Human Leukocyte Sterol Synthesis. *Atherosclerosis*, **35**, 41-49. http://dx.doi.org/10.1016/0021-9150(80)90026-X

[15] Slater, H.R. and Robertson, F.W. (1979) A Comparison of Delipidated Sera Used in Studies of Sterol Synthesis by Human Mononuclear Lenkocytes. *Journal Lipid Research*, **20**, 413-416.

[16] Cham, B.E., Kostner, K.M., Dwivedy, A.K., Shafey, T.M., Fang, N.X. and Mahon, M.G. (1995) Lipid Apheresis: An *in Vivo* Application of Plasma Delipidation with Organic Solvents Resulting in Acute Transient Reduction of Circulating Plasma Lipids in Animals. *Journal of Clinical Apheresis*, **10**, 61-69. http://dx.doi.org/10.1002/jca.2920100202

[17] Cham, B.E., Kostner, K.M., Dwivedy, A.K., Shafey, T.M., Fang, N.X. and Mahon, M.G. (1996) Lipid Apheresis in an Animal Model Causes *in Vivo* Changes in Lipoprotein Electrophoretic Patterns. *Journal of Clinical Apheresis*, **11**, 61-70. http://dx.doi.org/10.1002/(SICI)1098-1101(1996)11:2<61::AID-JCA2>3.0.CO;2-8

[18] Kostner, K.M., Smith, J.L., Dwivedy, A.K., Shafey, T.M., *et al.* (1997) Lecithin Cholesterol Acyltransferase Activity in Normocholesterolemic and Hypercholesterolemic Roosters: Modulation by Lipid Apheresis. *European Journal of Clinical Investigation*, **27**, 212-218. http://dx.doi.org/10.1046/j.1365-2362.1997.960643.x

[19] Groener, J.E.M., Van Rozen, A.E. and Erkelens, D.W. (1984) Cholesteryl Ester Transfer Activity: Localization and Role in the Distribution of Cholesteryl Ester among Lipoproteins in Man. *Atherosclerosis*, **50**, 261-271. http://dx.doi.org/10.1016/0021-9150(84)90074-1

[20] Cham, B.E., Kostner, K.M., Shafey, T.M., Smith, J.L. and Colquhoun, D.M. (2005) Plasma Delipidation Process Induces Rapid Regression of Atherosclerosis and Mobilization of Adipose Tissue. *Journal of Clinical Apheresis*, **20**, 143-153. http://dx.doi.org/10.1002/jca.20060

[21] Cham, B.E. and Smith, J.L. (1994) Lipid Apheresis in an Animal Model Causes Acute Reduction in Plasma Lipid Concentrations and Mobilization of Lipid from Liver and Aorta. *Pharmacology Life Science Advances*, **13**, 25-32.

[22] Cham, B.E. (1990) Autologous Plasma Delipidation Using a Continuous Flow System. US Patent No.: 4895558A.

[23] Cham, B.E., Kostner, K.M., Colquhoun, D.M. and Dwivedy, A.K. (1994) Lipid-Apheresis: An *in Vivo* Application of Delipidation but Apo Lipoprotein Retention in Plasma of Calves by a Continuous Extracorporeal Procedure. *Atherosclerosis*, **109**, 158. http://dx.doi.org/10.1016/0021-9150(94)93644-7

[24] Cham, B.E., Kostner, K.M., Colquhoun, D.M. and Dwivedy, A.K. (1994) Lipid-Apheresis: An *in Vivo* Application of Delipidation but Apo Lipoprotein Retention in Plasma of Pigs by a Continuous Extracorporeal Procedure. *Atherosclerosis*, **109**,159. http://dx.doi.org/10.1016/0021-9150(94)93645-5

[25] Sacks, F.M., Rudel, L.L., Conner, A., Keefe, A., Kostner, G., Baki, T., *et al.* (2009) Selective Delipidation of Plasma HDL Enhances Reverse Cholesterol Transport *in Vivo*. *Journal of Lipid Research*, **50**, 894-907. http://dx.doi.org/10.1194/jlr.M800622-JLR200

[26] Waksman, R., Torguson, R., Kent, K.M., Pichard, A.D., Suddath, W.O., Satler, L.F., *et al.* (2010) A First-in-Man, Randomized, Placebo-Controlled Study to Evaluate the Safety and Feasibility of Autologous Delipidated High-Density Lipoprotein Plasma Infusions in Patients with Acute Coronary Syndrome. *Journal of the American College Cardiology*, **55**, 2727-2735. http://dx.doi.org/10.1016/j.jacc.2009.12.067

[27] Nissen, S.E., Tsunoda, T., Tuzcu, E.M., Schoenhagen, P., *et al.* (2003) Effect of Recombinant Apo A-I Milano on Coronary Atherosclerosis in Patients with Acute Coronary Syndromes. A Randomized Controlled Trial. *JAMA*, **290**, 2292-2300. http://dx.doi.org/10.1001/jama.290.17.2292

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Does the Animal Origin Influence the Calcification of Xenograft Tissue Heart Valve Substitutes? Comparison between Bovine and Camel Pericardium in a Subcutaneous Rat Model

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Abstract

Objective: To validate the hypothesis that camel pericardium could be more protected than bovine pericardium against calcification process according to the huge difference in their respective life-style and lifetime. Methods: Glutaraldehyde (GA) fixed bovine and camel pericardium samples (BP and CP respectively) were both implanted in 30 New Zealand white rats (2 BP and 2 CP matched specimens in each animal) and explanted after 60 days. Unimplanted GA-fixed samples of both species served as control. Matched implanted samples and unimplanted samples were randomly submitted to elemental analysis by spectroscopy, phospholipid extraction, macroscopic and X-ray examination and histology. Results: At 60 days, calcium and phosphorus content were respectively 9.54% ± 3.1% and 4.79% ± 1.4% of tissue dry weight in BP, and 12.52% ± 2.7% and 6.14% ± 1.3% of tissue dry weight in CP (ns). In X-ray analysis, the calcification score was 1.28 ± 0.45 and 2.14 ±

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0.98 in BP and CP samples respectively without significant difference ($p < 0.08$). In histology, calcifications were lower in BP than in CP: 1.37 ± 0.85 vs 2.28 ± 0.83 (ns); collagen fibers were better conserved in BP than in CP: 2.4 ± 0.48 vs 1.87 ± 0.78 (ns), and less disoriented: 25% vs 62% (ns). In unimplanted samples, there was a higher but not significant rate of extracted lipids in CP: 5.7 ± 1.8 vs 9.5 ± 3.8 nanomoles in PS fraction and 11.3 ± 3.7 vs 19 ± 7.7 nanomoles in total fatty acids, in BP and CP samples respectively. All results were in conjunction and demonstrated a higher but not significant rate of mineralization in camel pericardium after implantation, which could be related to a higher but not significant basic rate of phospholipid and fatty acids. Conclusion: This experiment study in a subcutaneous rat model has failed to valid our hypothesis. Because the differences observed between bovine and camel pericardium did not reach the significance, at the best, there is no difference between both species and at the worst, camel pericardium has a higher rate of the phosphatidylserine fraction of phospholipid, and is more sensitive and prompt to calcification.

Keywords

Pericardium, Calcification, Bovine, Camel, Xenograft Tissue, Heart Valve Substitutes

1. Introduction

Structural dysfunction, due to calcification and non-calcific deterioration, is the major cause of failure of bioprosthetic heart valves. Glutaraldehyde (GA) was introduced for fixation of bioprosthetic valves to decrease immunogenecity, increase crosslinking of connective tissue proteins, and increase strength and durability [1]. Consequently, it has been used extensively for the preservation of bovine pericardium derived valves and porcine aortic valves. However, it has been found that GA fixation stimulates dystrophic calcification, as a consequence of xenograft cells devitalization and a toxic effect of unstable cross-linking. The mechanism of mineralization of GA-fixed bioprosthesis consists of attraction and precipitation in which activated phosphatases bind to calcium upon lipid-based cell debris, which are full of phospholipids [2] [3]. Despite the controversies around the different effects of GA on the calcification process [4] [5], GA-fixed tissue remains the basic concept of xenograft engineering and GA is the standard fixative with no alternative in view. Nevertheless, additional anticalcificant treatments have been developed to block calcification at the cellular level and to provide better structural integrity [6] [7].

Regardless of the fixation and the post-fixation treatments of the pericardium, we have considered that the animal origin of the xenograft could influence the process of calcification and we have tested this hypothesis in a comparison between bovine and camel origin. The main objective of this study was finding a potential difference in the degree of calcification for GA-fixed bovine (BP) and camel pericardium (CP) in a subcutaneous rat model. The organisms live a diverse lifestyle with a variance of eating habits, habitats and life expectancy which could involve variety of components present in BP and CP, being able to influence the calcification process of their respective tissue.

2. Material and Methods

2.1. Tissue Preparation

Samples of bovine pericardium (BP) and camel pericardium (CP) were obtained from a local abattoir. The removal of any adherent adipose tissue was followed by rinsing in 0.9% cold physiological saline. Samples were pre-fixed with a high concentration of glutaraldehyde (0.625%) for a period of 30 minutes. To remain consistent, using a round cutter instrument, the samples were assembled into 0.8 cm^2 sections. Subsequently, all samples were stored by immersion in 0.625% GA at 4˚C for 24 hours.

2.2. Subcutaneous Implantation

The round samples, both BP and CP, were implanted subcutaneously in thirty 2-week-old New Zealand white rats. The surgical procedure was conducted under general anesthesia by the effect of ether. The dorsal area was shaved and disinfected prior to implantation of tissue samples which were rinsed in saline for 3 min to remove

any residual storage solution. Each rat received 4 samples, 2 BP and 2 CP; each sample was implanted through an incision of 1 cm into the dorsal wall. The unimplanted remaining samples, BP and CP, were stored in 0.625% GA at 4˚C as a control group. All animals utilized in this research were cared for according to the policies and principles established by the Animal Welfare Act and the NIHGuide for Care and Use of Laboratory Animals. After 60 days, the animals were sacrificed using an overdose of barbiturates and the samples were retrieved and oriented to three different analysis. For elemental analysis, 10 samples, 5 BP and 5 CP, were stored under cold conditions. For lipid extraction and fatty acid analysis, 4 samples, 2 BP and 2 CP, were frozen using liquid nitrogen. For morphology and radiography, 10 other samples were collected, 5 BP and 5 CP, and then they were fixed in 4% formaldehyde for histologic analysis. The BP and CP samples explanted were matched by animals to avoid any variations due the hosts and were identified numerically. Concurrently, the unimplanted and stored during the same 60 days BP and CP samples were rinsed in saline for 3 min to remove any residual storage solution, and then were oriented to the different analysis, as the explanted samples; they were identified as 0 (BP0, CP0).

2.3. Elemental Analysis

Calcium contents were assessed by atomic absorption spectroscopy. Samples were dried to a content weight in a desiccator oven and hydrolyzed with HNO_3 (0.75 mol/l) at 68˚C for 15 hours. After centrifuging at 2500×g, the fluid was removed, diluted and the calcium content was determined using the atomic absorption spectrometer UNICAM Solaar 989. A calibration curve was plotted using a set of crescent concentrations of a calcium standard solution and distilled water blank. Calcium contents, expressed as percentage of tissue dry weight, represent the mean of at least three different spectrometric determinations. Phosphorous contents, expressed, as percentage of tissue dry weight, were assessed by inductively coupled analysis.

2.4. Phospholipid Analysis

The lipids and then the phospholipids were extracted and separated into different classes. The phosphatidyl serine fraction (PS) was transesterified and the fatty acid methyl esters were analyzed by gas chromatography. Each fatty acid is expressed in % relative to the total fatty acids in the fraction PS.

2.5. Macroscopic Examination and X-Ray Analysis

The morphology of each sample was assessed before submission to X-ray analysis using mammography technique. For X-ray analysis the presence of calcium deposits was quantified on the basis of a 0 - 4 score as follows: 0 = absent; 1 = focal, pinpoint, <1 mm of diameter; 2 = focal, >1 mm of diameter or pinpoint multiple; 3 = multiple >1 mm diameter; 4 = massive deposition.

2.6. Histology

The pericardial samples, BP and CP, were fixed in 4% formaldehyde, processed, and stained with hematoxylin-eosin (H&E) and von Kossa staining. The following parameters were evaluated:

- Presence, amount and type of mineralization (nodular and laminar, whether calcium deposits occurred on cells, collagen fibers or both) on Von Kossa stained sections.
- Presence, amount and type of inflammatory reaction (either granulomatous, lymphocytic or neutrophilic) on hematoxylin-eosin stained sections.

A semi quantitative method was applied on histologic sections to evaluate the amount of mineralization and inflammatory infiltrate: (0) absent; (1) minimal; (2) mild; (3) moderate; and (4) severe.

2.7. Statistical Analysis

Quantitative data were presented as mean ± SEM. The statistical analysis included a variance check (ANOVA); The nonparametric Mann-Whitney U and Wilcoxon rank sum tests were used with a pairwise comparison of 95% confidence intervals. The probability was set at $p < 0.05$ for significance.

3. Results

All animals except one survived to the surgical procedure and they could be sacrificed at 60-day follow-up.

Implanted pericardium samples were matched by animal and then randomly distributed to the different analyses. Unimplanted samples were randomly distributed to the different analyses after storage.

3.1. Elemental Analysis

In unimplanted samples, calcium and phosphorus contents were at the limit of detection without difference between bovine and camel origin (**Table 1**). In implanted samples, calcium and phosphorus contents ranged from 4.22% to 15.93% and 2.33% to 7.70% respectively. In matched implanted samples, calcium and phosphorus contents were higher in camel pericardium (**Table 1**) showing a higher mineralization process in this group. However, the difference between BP and CP were not significant (**Figure 1**).

3.2. Phospholipid Analysis

The phospholipid analysis was done on 8 unimplanted samples (4 BP and 4 CP) and 4 matched implanted samples (2 BP and 2 CP). In unimplanted bovine and camel samples, the phosphatidylserine fraction ranged from 4.6 to 8.5 Nanomoles and from 4.6 to 15.2 Nanomoles respectively, and the total fatty acids in PS fraction, after new calibration, ranged from 6.9 to 16.9 Nanomoles and from 9.2 to 30.4 Nanomoles respectively, showing a higher rate of extracted lipids in camel samples: in mean, 5.7 ± 1.8 vs 9.5 ± 3.8 (ns) Nanomoles in PS fraction and 11.3 ± 3.7 vs 19 ± 7.7 (ns) Nanomoles in total fatty acids, in bovine and camel samples respectively. However, the distributions of the fatty acids were comparable between camel and bovine pericardium samples (**Figure 2**). In implanted samples, results were similar: in mean, 3.5 ± 1 vs 16.8 ± 8.5 (ns) Nanomoles in PS fraction and 7.1 ± 2 vs 33.6 ± 17 (ns) Nanomoles in total fatty acids, in bovine and camel samples respectively with a comparable distribution of fatty acids (**Figure 2**). Interestingly, PS fraction and total fatty acids were in implanted samples lower in BP and higher in CP in comparison with respective unimplanted samples.

Table 1. Calcium and Phosphorous contents in bovine (BP) and camel (CP) pericardium matched samples. 0 indicates unimplanted samples; 1 to 5 indicate implanted samples. Results are expressed in % of tissue dry weight.

Content	Samples	0	1	2	3	4	5	Mean
Calcium	BP	<300 ppm	8.59	11.21	10.12	13.54	4.22	9.54 ± 3.1
	CP	<300 ppm	13.62	12.04	7.71	13.32	15.93	12.52 ± 2.7
Phosphorous	BP	0.09	4.34	5.61	5.01	6.64	2.33	4.79 ± 1.4
	CP	0.10	6.66	5.93	3.81	6.49	7.70	6.14 ± 1.3

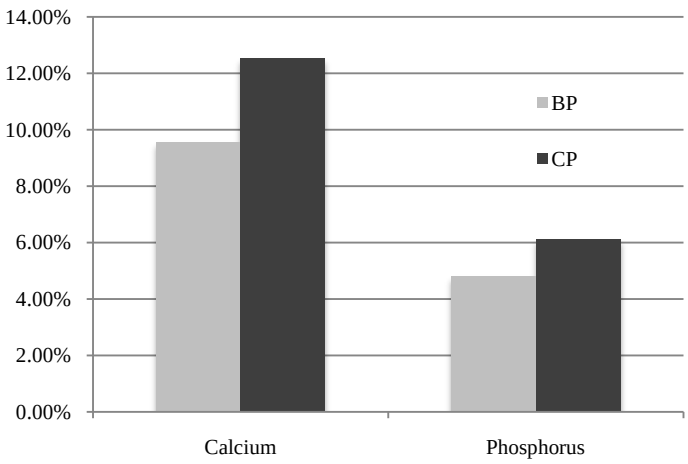


Figure 1. Histogram of calcium and phosphorous content expressed as percentage tissue dry weight. BP, bovine pericardium, CP, camel pericardium.

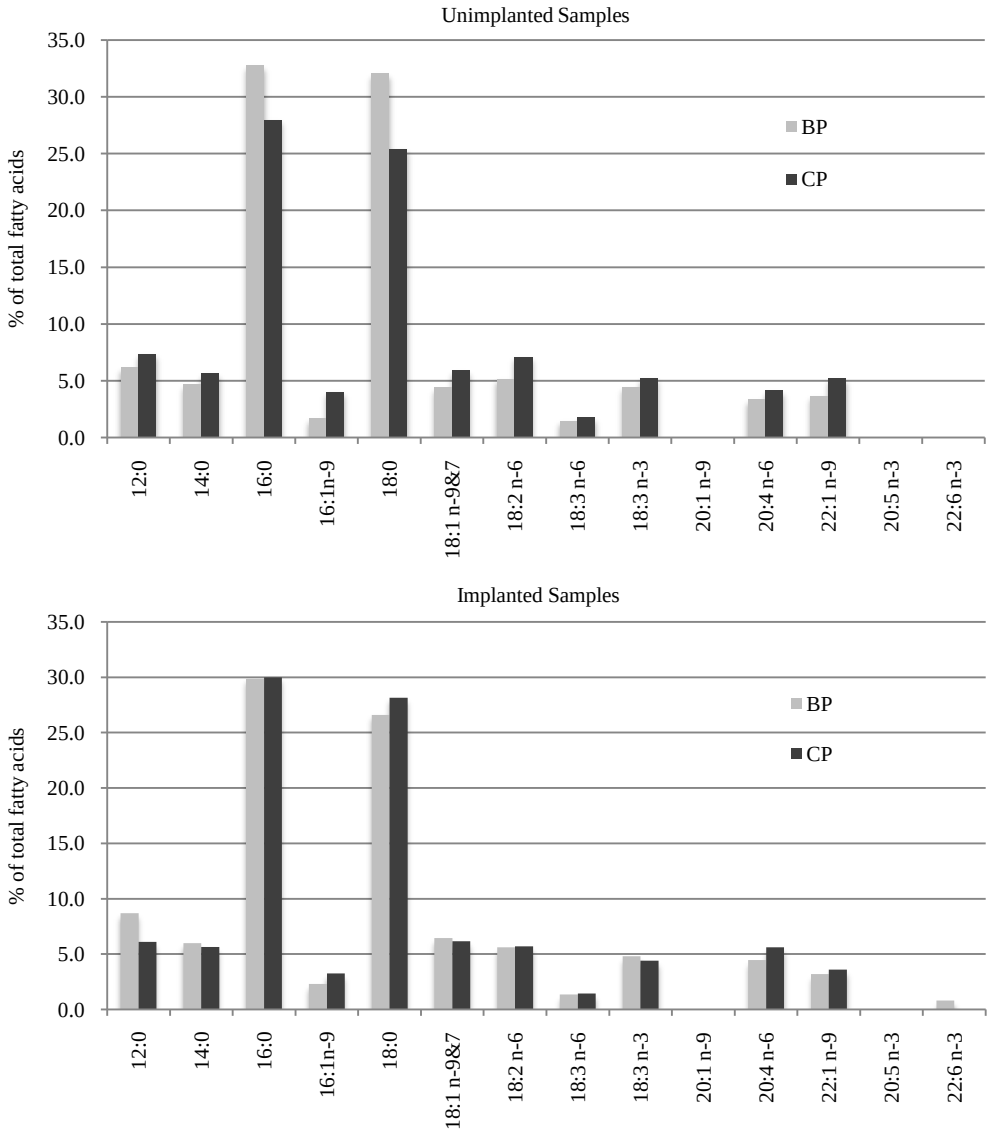


Figure 2. Histogram of means for fatty acids present in unimplanted and implanted BP and CP samples. BP, bovine pericardium, CP, camel pericardium.

3.3. Macroscopic Examination and X-Ray Analysis

The macroscopic aspect of unimplanted and stored samples looks like fine; all are soft, supple, nongritty and well preserved without obvious difference between bovine and camel samples (**Figure 3(a)** & **Figure 3(b)**). On the contrary, all implanted samples look like small bones, more and less rigid, thickened, hard and gritty, as the consequence of the mineralization and calcification process during the implantation period; there was no visible difference between bovine and camel samples (**Figure 3(c)** & **Figure 3(d)**).

The X-ray analysis involved 14 matched implanted samples (7 BP and 7 CP); the presence of calcium deposit was quantified on the basis of a 0 - 4 score (**Figure 4**) by two independent observers. The calcification score was 1.28 ± 0.45 and 2.14 ± 0.98 in BP and CP samples respectively without significant difference ($p < 0.08$).

3.4. Histology

Histological analysis involved 4 unimplanted samples (2 BP and 2 CP). There was no difference in the cell components: all pericardium samples had absence of lymphocytes, neutrophils, giant cells and calcification. The

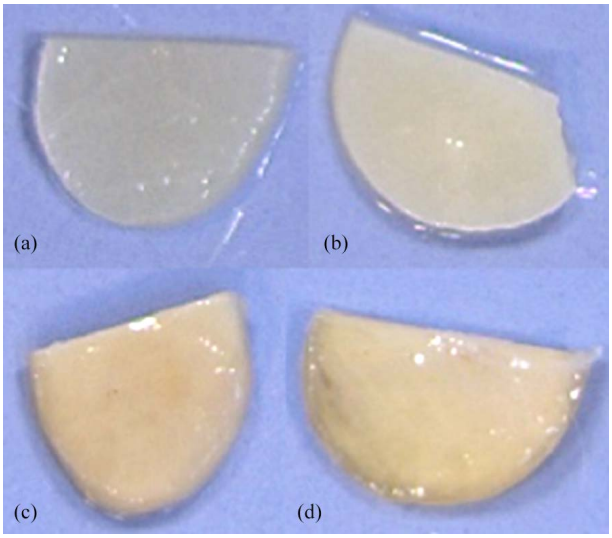
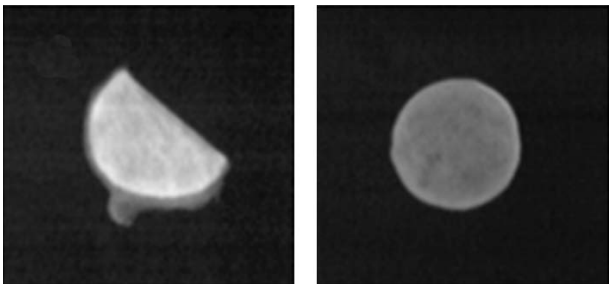


Figure 3. Morphology of pericardium samples before specific analysis. (a) BP unimplanted sample (b) CP unimplanted sample (c) BP implanted sample (d) CP implanted sample. BP, bovine pericardium, CP, camel pericardium.



(a) (b)

Figure 4. Mammography X-ray analysis of implanted samples. (a) shows a CP implanted sample with obvious calcium deposits with a score of 4. (b) shows a CP implanted sample with calcifications score of 1.BP, bovine pericardium, CP, camel pericardium.

collagen fibers were present at a score of 3 and were well oriented in all samples (**Figure 5**, IV).

Histological analysis involved 16 matched implanted samples (8 BP and 8 CP). The different microscopic aspects observed are illustrated in **Figure 5** and semi-quantitative scores are summarized in **Figure 6**. Calcifications were lower in BP than in CP: 1.37 ± 0.85 vs 2.28 ± 0.83 (ns); collagen fibers were better conserved in BP than in CP: 2.4 ± 0.48 vs 1.87 ± 0.78 (ns) and less disoriented: 25% vs 62% (ns). The inflammatory infiltration by lymphocytes and giant cells was overall low with a light difference between BP and CP (ns).

4. Comments

Calcification is an important factor in clinical dysfunction of bioprosthetic heart valves. The onset of calcification has been postulated to originate from an electrostatic attraction between the acid phospholipids of the connective tissue and calcium. Accelerated calcification is associated with young recipient age, glutaraldehyde (GA) fixation, and high mechanical stress. Xenograft heart valves treated with GA and mounted in a rigid stent are the most widely used cardiac bioprostheses. Although stable cross-links in cellular and extracellular matrix proteins are considered to reduce immunogenicity, GA treatment has been demonstrated to increase calcification by fixing cellular debris in place [1] [2].

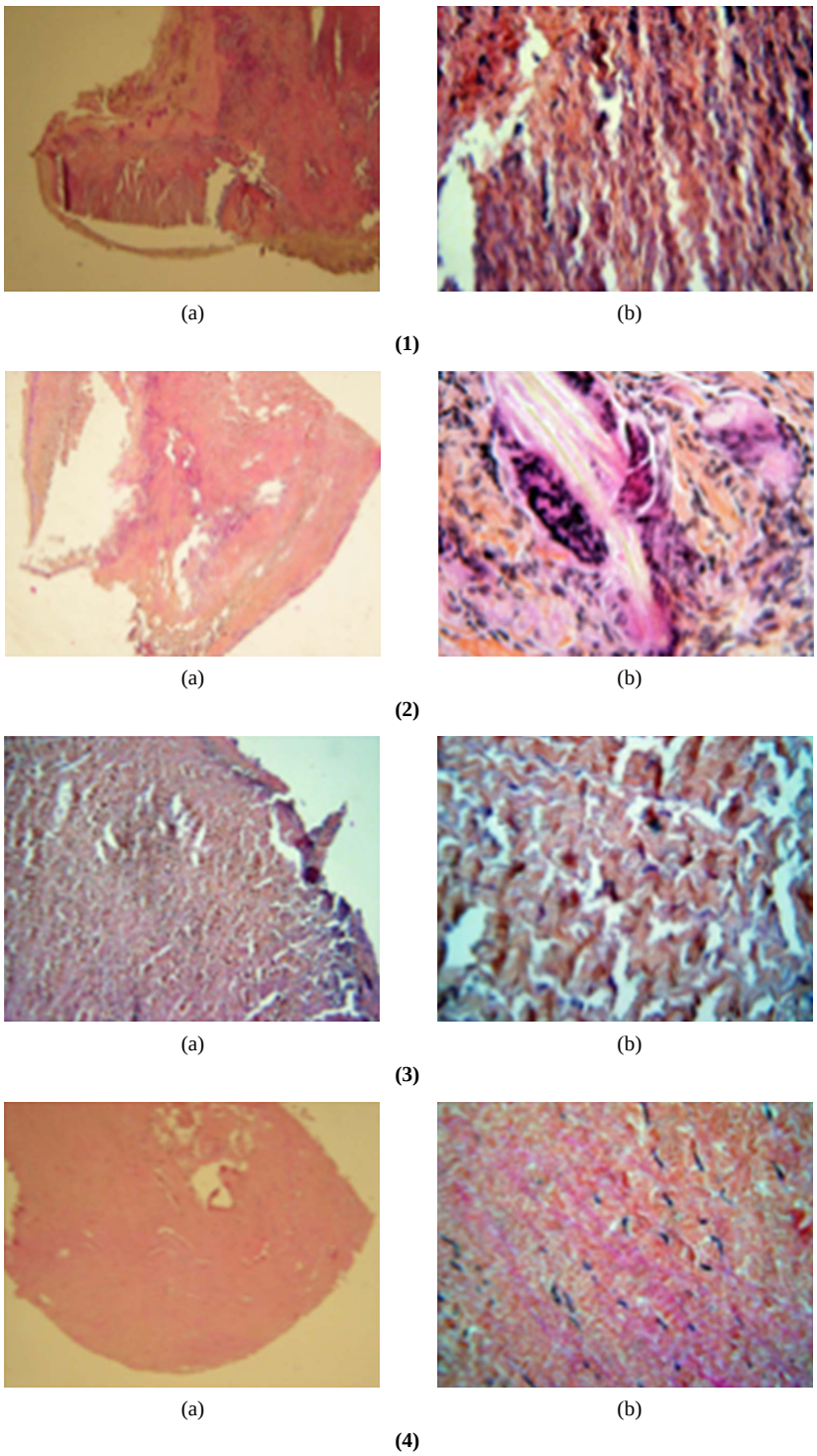


Figure 5. Histology of pericardium samples. (1) Implanted camel pericardium (a) Light microscopy in stained with H&E showing mild disruption of collagen fibers of the pericardial matrix; (b) High microscopy with Von Kossa staining showing severe calcifications (brown deposits). (2) Implanted bovine pericardium stained with H&E, (a) Light microscopy showing the presence of inflammatory cells (lymphocytes and giant cells); collagen fibers of the pericardial matrix remaining well oriented; (b) High microscopy showing giant cells. (3) Implanted camel pericardium with Von Kossa staining (a) Light microscopy and (b) High microscopy showing severe disruption of collagen fiber of the pericardial matrix with severe calcification (brown deposits). (4) Unimplanted camel pericardium (a) stained with H&E showing a normal pericardial matrix with well-oriented collagen fibers and without inflammatory cell; (b) Von Kossa staining showing no calcification.

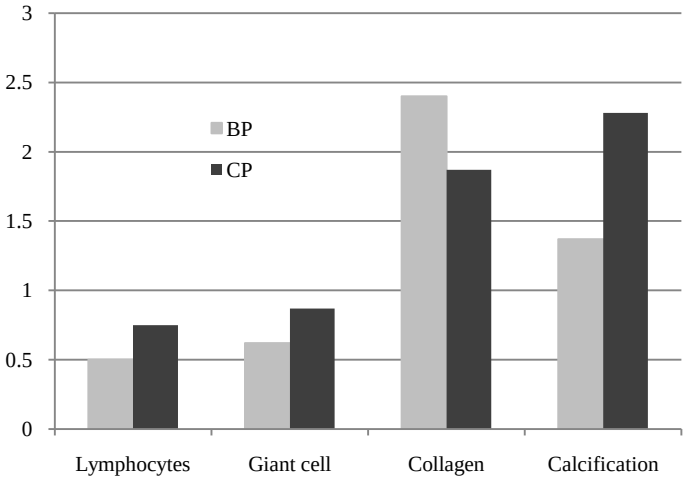


Figure 6. Histogram of mean scoring for microscopic examinations of implanted samples. BP, bovine pericardium, CP, camel pericardium.

Cells are the predominant location of mineralization and the usual pretreatment of commercially available bioprostheses with GA, done to improve tissue durability, also potentiate calcification [4] [8]. GA fixation devitalizes but does not remove connective tissue cells that are prone to be the initial site of calcium deposition. Calcification of the extracellular matrix structural proteins collagen and elastin has been studied; collagen and elastic fibers can serve as nucleation sites for calcium phosphate minerals, independent of cellular components [9].

Tremendous efforts have been made to solve this problem and numerous experiments have been carried out to investigate different calcium mitigating agents. Nowadays, all new anticalcificant treatments aim at washing residual GA out; thereafter, either additional surfactants are applied or methods to block calcification at the cellular level or to extract cells are used [6] [10]. At present, not all details of the different treatments are fully disclosed.

Significant correlations between phospholipid levels and calcification have been well established and phospholipids appear to be a significant contributor to the calcification of bioprosthetic tissue [2] [3]. Other mechanisms may also contribute to the observed calcification, as residual aldehyde toxicity and mechanical and cellular factors. Different lipoprotein profiles, potential differences in calcium metabolism, hematologic differences may play a role in the onset of calcification [11] [12].

The animal origin of the xenograft could also influence the process of calcification [13]. We have tested this hypothesis in a comparison between bovine and camel origin. The organisms live a diverse lifestyle with a variance of eating habits, habitats and life expectancy which could involve variety of components present in BP and CP, able to influence the calcification process of their respective tissue. The difference in lifestyle between the two species is the huge, that a significant difference in the calcification process of pericardium could be expected. This study used the subcutaneous implantation of specimens. The subcutaneous model has emerged as a technically convenient and economically advantageous vehicle for investigating host and implant determinants and mechanisms of mineralization, as well as for screening potential strategies for inhibition of calcification [11] [13] [14]. The rat subcutaneous implant is a favorable low cost model with easy availability.

In our experiments, a difference in rate of calcification of the implanted specimens between bovine and camel pericardium origin was observed. The calcium measurements using spectroscopy analysis were in conjunction with the X-ray analysis and the histological score of calcification of samples: all results demonstrated a higher rate of mineralization in camel pericardium specimens. The phospholipid analysis was focused on phospatidylserine fraction and corresponding fatty acids in unimplanted samples, showing higher levels in camel pericardium, with a similar distribution of fatty acids. All differences observed between bovine and camel pericardium did not reached the level of significance, probably as a consequence of the limited number of specimens analyzed. However, even if the results observed were not in the expected direction, this study has confirmed that donor species could influence calcification of tissue valve substitutes [13] and correlation between phospholipid levels and mineralization [3]. Only Phospatidylserine fraction was analysed because it has been described as the

main phospholipid involved in the process of calcification [1] and also to limit the cost of the study. Interestingly, the distributions of fatty acids were similar in implanted and unimplanted samples, and in bovine and camel origin, showing that volume of phospholipid is maybe more determinant than specificity in the process of calcification. However, it is well established that additional treatment to remove phospholids from the xenograft tissue or to neutralizes the toxic residual aldhehyde groups have failed to prevent mineralization; the role of residual matrix immunoreactivity could be predominant.

Tissue engineering heart valves constructed from a xenogeneic or an allogeneic heart valve scaffold eventually covered with autologous endothelial cells are considered to overcome all these obstacles [10] [15]. Several studies have led to the selection of human allograft, not xenografts, as a basis for decellularization technologies designed to obtain functioning valve extracellular matrix (ECM) scaffolds for tissue engineering of heart valves, a goal which involves a unique combination of biological, engineering, and technological hurdles [12] [15]. The tissue engineering heart valve is the prototype multidisciplinary collaborative project that is virtually ideal as the archetype for the new era of bioengineered solutions to complex cardiac diseases.

A limitation of the study is a lack of investigation on mechanical properties like elasticity and strength as well as resistance to enzymatic and cellular digestion. Such investigations would have been planned if this study had been able to demonstrate a lower mineralization process in camel pericardium and so a possible benefit to develop its use.

5. Conclusion

Calcification and deterioration of GA-fixed heart valve substitutes remain a concern. We made the hypothesis that camel pericardium could be more protected than bovine pericardium against calcification according to the huge difference in their respective lifestyle and lifetime. Our experiment study in a subcutaneous rat model has failed to valid this hypothesis. Because the differences observed between bovine and camel pericardium did not reach the significance, at the best, there is no difference between both species and at the worst, camel pericardium is more sensitive and prompt to calcification, with a higher rate of the phosphatidylserine fraction of phospholipid. These observations led us to interrupt our research in camel tissue substitute and to orient them to tissue engineering heart valves which could be more promising according to the lower impact of immunological and enzymatic reactions.

References

[1] Schoen, F.J. and Levy, R.J. (2005) Calcification of Tissue Heart Valve Subsitutes: Progress toward Understanding and Prevention. *The Annals of Thoracic Surgery*, **79**, 1072-1080. http://dx.doi.org/10.1016/j.athoracsur.2004.06.033

[2] Levy, R.J. (1994) Glutaraldehyde and the Calcification Mechanism of Bioprosthetic Heart Valves. *The Journal of Heart Valve Disease*, **3**, 101-104.

[3] Cunanam, C.M., Cabiling, C.M., Dinh, T.T., Shen, S.S., Tran-Hata, P., Rutledge, J.H. and Fishbein, M.C. (2001) Tissue Characterization and Calcification Potential of Commercial Bioprosthetic Heart Valves. *The Annals of Thoracic Surgery*, **71**, S417-S421. http://dx.doi.org/10.1016/S0003-4975(01)02493-6

[4] Gong, G., Ling, Z., Seifter, E., Factor, S.M. and Frater, R.W.M. (1991) Aldehyde Tanning: The Villain in Bioprosthetic Valve Calcification. *European Journal Cardio-Thoracic Surgery*, **5**, 288-293. http://dx.doi.org/10.1016/1010-7940(91)90037-K

[5] Vincentelli, A., Latremouille, C., Zegdi, R., Shen, M., Lajos, P.S., Chachques, J.C. and Fabiani, J.N. (1998) Does Glutaraldehyde Induce Calcification of Bioprosthetic Tissues? *The Annals of Thoracic Surgery*, **66**, S255-S258. http://dx.doi.org/10.1016/S0003-4975(98)01098-4

[6] Walter, T., Falk, V., Autschbach, R., Diegeler, A., Rauch, T., Weigl, C., Gunther, B., van Son, J.A. and Mohr, F.W. (1998) Comparison of Different Anticalcification Treatments for Stentless Bioprostheses. *The Annals of Thoracic Surgery*, **66**, S249-S254. http://dx.doi.org/10.1016/S0003-4975(98)00985-0

[7] Carpentier, S.M., Chen, L., Shen, M., Fornes, P., Martinet, B., Quintero, L.J., Witzel, T.H. and Carpentier, A.F. (1998) Heat Treatment Mitigates Calcification of Valvular Bioprostheses. *The Annals of Thoracic Surgery*, **66**, S264-S266. http://dx.doi.org/10.1016/S0003-4975(98)01101-1

[8] Hoffmann, D., Gong, G., Liao, K., Macaluso, F., Nikolic, S.D. and Frater, R.W.M. (1992) Spontaneous Host Endothelial Growth on Bioprostheses. Influence of Fixation. *Circulation*, **86**, II75-II79.

[9] Hopkins, R.A. (2005) Tissue Engineering of Heart Valves. Decellularized Valve Scaffolds. *Circulation*, **111**, 2712-2714. http://dx.doi.org/10.1161/CIRCULATIONAHA.104.527820

[10] Schoen, F.J., Levy, R.J., Hilbert, S.L. and Bianco, R.W. (1992) Antimineralization Treatments for Bioprosthetic Heart Valves. *The Journal of Thoracic and Cardiovascular Surgery*, **104**, 1285-1288.

[11] Neethling, W.M., Glancy, R. and Hodge, A. (2010) Mitigation of Calcification and Cytotoxicity of a Glutaraldehyde Preserved Bovine Pericardial Matrix: Improved Biocompatibility after Extended Implantation in the Subcutaneous Rat Model. *The Journal of Heart Valve Disease*, **19**, 778-785.

[12] Rieder, E., Seebacher, G., Kasimir, M.T., Eichmair, E., Winter, B., Dekan, B., Wolner, E., Simon, P. and Weigel, G. (2005) Tissue Engineering of Heart Valves. Decellularized Porcine and Human Valve Scallops Differ Importantly in Residual Potential to Attract Monocytic Cells. *Circulation*, **111**, 2792-2797. http://dx.doi.org/10.1161/CIRCULATIONAHA.104.473629

[13] Carpentier, S.M., Monier, M.H., Shen, M. and Carpentier, A.F. (1995) Do Donor or Recipient Species Influence Calcification of Bioprosthetic Tissues? *The Annals of Thoracic Surgery*, **60**, S328-S331. http://dx.doi.org/10.1016/0003-4975(95)00244-F

[14] Pettenazzo, E., Valente, M. and Thiene, G. (2008) Octanediol Treatment of Glutaraldhehyde Fixed Bovine Pericardium: Evidence of Anticalcification Efficacy in the Subcutaneous Rat Model. *European Journal Cardio-Thoracic Surgery*, **34**, 418-422. http://dx.doi.org/10.1016/j.ejcts.2008.05.012

[15] Mendelson, K. and Schoen, F.J. (2006) Heart Valve Tissue Engineering: Concepts, Approaches, Progress, and Challenges. *Annals of Biomedical Engineering*, **34**, 1799-1819. http://dx.doi.org/10.1007/s10439-006-9163-z

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