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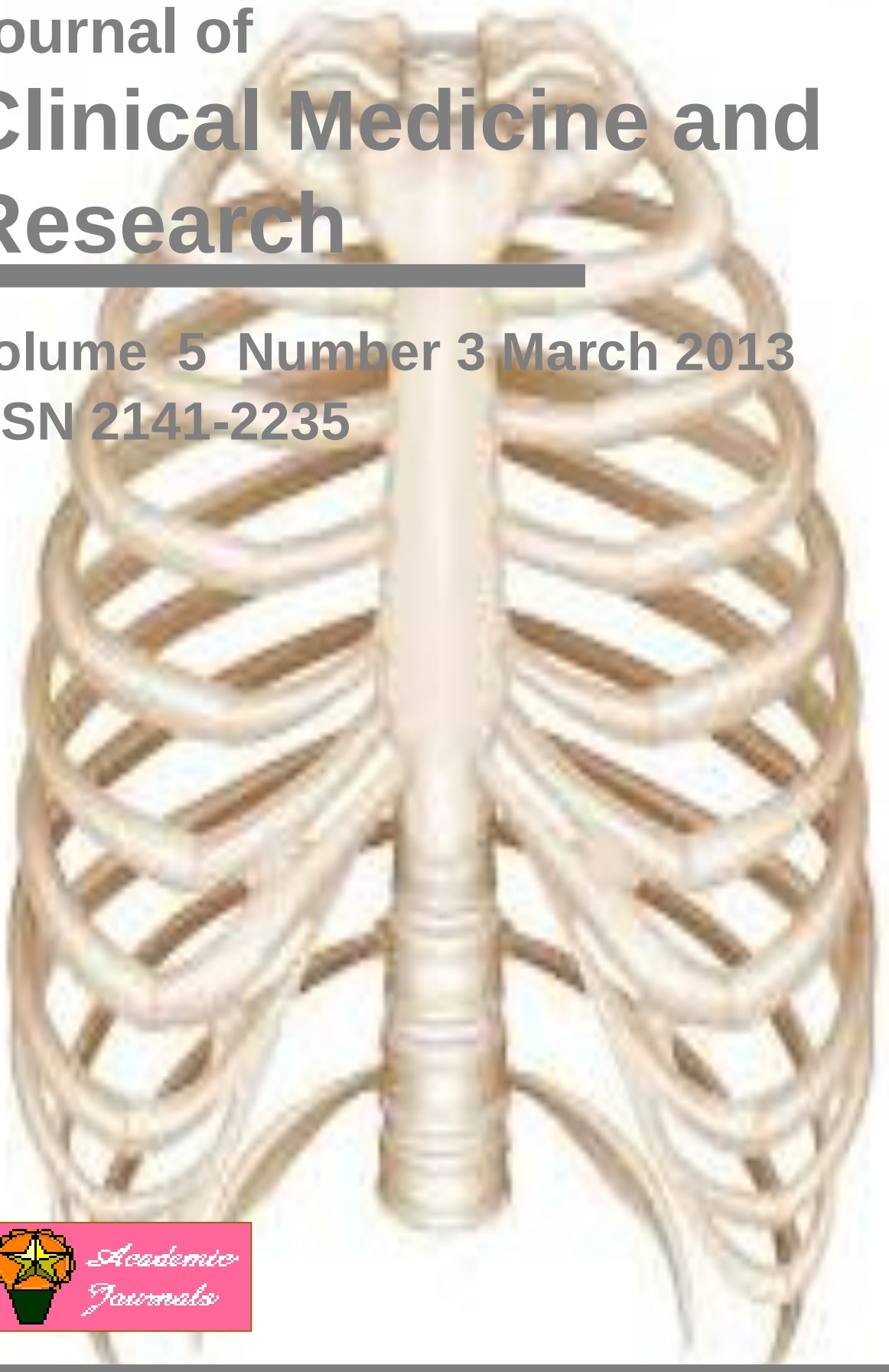

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Nishimura (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 2001), (Chege, 1998; Stein, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
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Giesielski SD, Seed TR, Ortiz JC, Melts J (2001). Intestinal parasites among North Carolina migrant farm workers. Am. J. Public Health. 82: 1258-1262

Stoy N, Mackay GM, Forrest CM, Christofides J, Egerton M, Stone TW, Darlington LG (2005). Tryptophan metabolism and oxidative stress in patients with Huntington’s disease. N. J. Neurochem. 93: 611–623.

Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. Parasitol. 116: 85-93.0.

Fransiscus RG, Long JC, (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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Research Articles

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Full Length Research Paper

Coronary artery bypass grafting and lung lobectomy: Functional outcomes at discharge

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Mortality and complication rates after on-pump versus off-pump coronary artery bypass grafting (CABG), and open lung lobectomy (OL) versus video-assisted lobectomy (VAL), have previously been reported, but further research regarding functional outcomes is needed. This study compared functional recovery at discharge between patients who underwent on-pump versus off-pump CABG, and OL versus VAL. In patients discharged during July to December, from 2006 to 2010, functional recovery was measured by comparing the Barthel index at admission and discharge. Complication rates and functional deterioration were compared in propensity score-matched groups of patients aged over 15 years who underwent isolated CABG of two or more arteries or lobectomy, and survived until discharge. The study included 3,901 on-pump CABG patients, 3,672 off-pump CABG patients, 6,029 OL patients, and 14,378 VAL patients. Patient and hospital characteristics, comorbidities, and preoperative care procedures were associated with on-pump versus off-pump CABG, and OL versus VAL, but functional deterioration was not. The complication rate was lower after VAL than OL. Dependent functional status at admission was associated with functional deterioration in patients who underwent lobectomy. Multidisciplinary treatment strategies to maintain functional status should be developed, and appropriate indications for lobectomy according to functional status at admission should be determined.

Key words: Coronary artery bypass grafting, complications, functional outcomes, lung lobectomy.

INTRODUCTION

With the increasing size of the elderly population, the number of disabled elderly individuals is increasing. According to the Organization for Economic Co-operation and Development (OECD) StatExtracts 2011, the proportion of the Japanese population aged over 65 years increased from 9.1% in 1980 to 23.1% in 2010 (for the United States, this proportion increased from 11.3 to 13.0% during the same time period) (OECD StatExtracts, 2011). As a growing number of elderly individuals develop cardiovascular and lung diseases, there is an increasing need for treatments that are less invasive and provide

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better functional outcomes. Previous studies have compared postoperative complication and mortality rates between patients who underwent on-pump versus off-pump coronary artery bypass grafting (CABG), and open lung lobectomy (OL) versus video-assisted lobectomy (VAL) (Angelini et al., 2009; Gopaldas et al., 2010; Kapetanakis et al., 2008; Kiernan et al., 2011; Kuss et al., 2010; Lim et al., 2006; Mishra et al., 2006; Motallebzadeh et al., 2006; Rivera et al., 2011).

Although the usefulness of off-pump CABG and VAL have been validated in randomized studies of selected subjects, it is desirable to conduct community-based studies to confirm the safety and effectiveness of minimally invasive surgery (MIS) procedures (off-pump CABG and VAL) versus conventional procedures (on-pump CABG and OL), to assist in determining appropriate procedure choices, and in implementing relevant healthcare policies (Kiernan et al., 2011; Kuss et al., 2010; Lim et al., 2006; Mishra et al., 2006; Rivera et al., 2011).

Previous studies used variable designs and definitions of disease severity (Kuss et al., 2010; Lim et al., 2006). The effects of selection bias have also been discussed (Gopaldas et al., 2010; Kiernan et al., 2011; Mishra et al., 2006; Rivera et al., 2011). Outcomes have been investigated in terms of mortality and complication rates, but functional recovery at discharge has not been sufficiently investigated (Angelini et al., 2009; Kapetanakis et al., 2008; Motallebzadeh et al., 2006). Our Japanese database which includes both functional status information and procedure-based severity information, enables comparisons of functional recovery between propensity score-matched groups of patients who underwent MIS versus conventional procedures. Analysis of such a large population-based database can help to determine the appropriate indications for surgical procedures.

This study used the Japanese administrative database, including data describing functional status at admission and discharge, and the dates and quantities of medical care items used to compare functional outcomes between on-pump versus off-pump CABG and OL versus VAL.

MATERIALS AND METHODS

Database

This was a secondary data analysis embedded in a government research project to develop the Japanese case-mix classification. In cooperation with the Ministry of Health, Labour and Welfare (MHLW), our research team started this project in 2001, using the administrative database for 2001 and 2002 to profile hospital performance and develop the payment system. All 82 academic hospitals in Japan participated in this project, and the number of participating community hospitals increased from 92 in 2003 to 1,650 in 2010 (Ministry of Health, Labour and Welfare, 2011). The database includes clinical data as well as claims data such as the date, charge, and quantity of medical care items used. The data of patients discharged between July 1 and October 31, during 2002 through 2005, and between July 1 and December 31 during 2006 through 2009, were collected and merged into a standardized electronic format by the MHLW. Original database included 13,604,026 patients from the 1,101 acute care hospitals participating in our project from 2004 to 2010. The study protocol was approved by the Ethics Committee of the University of Occupational and Environmental Health, Fukuoka.

Study patients

Of the 12,502,528 patients discharged during the 5 years from 2006, 26,472 patients who underwent coronary artery surgery (CABG or intraoperative angioplasty) in 408 hospitals, and 84,564 patients who underwent lung resection (wedge resection, partial lobectomy, lobectomy, or sleeve resection) in 900 hospitals, were identified. Patients who underwent CABG of two or more coronary vessels (22,506 patients in 404 hospitals), or resection of one or more lobes with or without sleeve resection (54,462 patients in 774 hospitals), were included in this study. Next, we enrolled the patients who were discharged from the hospitals participating in our project during the 5 consecutive years, from 2006 in this study. Patients aged less than 15 years, who had missing barthel index (BI) data, and who underwent aneurysmectomy or valve surgery during the same hospitalization were excluded.

Definitions of variables

The following variables were compared between on-pump and off-pump CABG, and between OL and VAL: age, sex, ambulance use, functional status measured by the BI, weighted comorbidity score measured by the Charlson comorbidity index (CCI), complication rates, length of intensive care unit (ICU) stay, percutaneous coronary intervention (PCI), critical care procedures, blood transfusion, chemoradiation therapy, operating room (OR) time, hospital patient volume, hospital teaching status (academic or community) and fiscal year (FY).

The critical care procedures recorded were pre- and post-operative mechanical ventilation, blood purification (hemodialysis, hemodiafiltration, or hemadsorption), and use of cardiac support devices (intra-aortic balloon pump, percutaneous cardiopulmonary support, or ventricular assist system). Preoperative critical care procedures and blood transfusion were used as markers of preoperative organ failure and anemia, respectively. PCI included pre- and postoperative thrombolysis, balloon angioplasty, stent insertion, and atherectomy because hybrid coronary artery revascularization was advocated (Holzhey et al., 2008). OR time included the time to implement monitoring, induce anesthesia, position the patient, and perform surgery.

Functional change was measured by the change in BI during hospitalization in patients who survived to discharge. The BI measures the ability performance in 10 activities of daily living (feeding, grooming, bathing, dressing, bowel and bladder care, toilet use, ambulation, transfers, and stair climbing) on a five-point scale, with scores ranging from 0 (totally dependent) to 100 (fully independent) (Sulter et al., 1999). The BI at admission was categorized as dependent (< 59), partially independent requiring assistance (60 to 84), or nearly completely independent (≥ 85), because it was expected that change in functional status during hospitalization would be associated with the functional status at admission (Kugler et al., 2003). Functional outcome was defined as BI at discharge minus BI at admission, and was categorized as improvement, no change, or deterioration (Kugler et al., 2003).

Age was divided into three categories: 15 to 64, 65 to 74, and ≥ 75 years. Up to four comorbidities and four complications were recorded per patient, and were indexed according to the International Classification of Diseases (ICD), 10th edition. The severity of chronic comorbid conditions was assessed using the

CCI (Sundararajan et al., 2004). Minor and major complications were examined. Minor complications were classified as present if the ICD codes corresponded with wound complications, hematoma and laceration, or disruption of organs by instrumentation or manipulation (T81 to T87) (Zhan and Miller, 2003). Major complications were defined as complications requiring reoperation for hemostasis and evacuation of hematoma, or intra-thoracic abscess. As information regarding surgeon experience was not recorded, hospital CABG and lung resection volumes were averaged over 5 years and divided into three groups: high-volume hospitals (HVHs), medium-volume hospitals (MVHs), and low-volume hospitals (LVHs); so that the three groups consisted of relatively equal numbers of patients (CABG: 105 LVHs, ≤ 32/year; 40 MVHs, 33 to 56/year; 25 HVHs, ≥ 57/year. Lung lobectomy: 197 LVHs, ≤ 61/year; 50 MVHs, 62 to 103/year; 31 HVHs, ≥ 104/year). Since 2006, hospital fee calculations have depended on the functional status of patients according to the BI which was recorded every day by well-trained nurses and audited by the MHLW.

Statistical analysis

Categorical data were compared between the on- and off-pump CABG groups and between the OL and VAL groups using the Chi-square test. Continuous variables were compared between MIS and conventional procedures using analysis of variance. Logistic regression models were used to identify recorded preoperative variables associated with the choice of on-pump versus off-pump CABG, and OL versus VAL. Propensity score-matched cohorts with equal proportions of patients likely to receive on-pump versus off-pump CABG, or OL versus VAL, were selected. Postoperative critical care procedures, blood transfusion, complication rates, and deterioration in BI were compared between these cohorts. Logistic regression models were also used to analyze associations between MIS versus conventional procedures, and complication rates or deterioration in BI. The model for analysis of complication rates included preoperative critical care procedures, deterioration in BI, and overall critical care procedures. Statistical analyses were performed using International business machines- Statistical package for the social sciences (IBM-SPSS) version 19.0. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Of all the patients reviewed in 263 hospitals, 3,901 on-pump CABG patients in 164 hospitals (692 in 36 academic hospitals), 3,672 off-pump CABG patients in 152 hospitals (1,126 in 34 academic hospitals), 6,029 OL patients in 225 hospitals (1,759 in 36 academic hospitals), and 14,378 VAL patients in 242 hospitals (4,975 in 37 academic hospitals) were identified. Comparisons of patient characteristics between groups showed that the categories of age, sex, and BI at admission were significantly different between patients who underwent on-pump versus off-pump CABG, and the categories of sex and CCI were significantly different between patients who underwent OL versus VAL. Comparisons of hospital characteristics between groups showed that there were significant differences in the categories of hospital patient volume and hospital teaching status between patients who underwent on-pump versus off-pump CABG, and OL versus VAL (Table 1).

Preoperative critical care, ICU care, blood transfusion, and mechanical ventilation were more frequent in patients who underwent on-pump versus off-pump CABG. ICU care, blood transfusion, mechanical ventilation, and chemoradiation therapy were more frequent in patients who underwent OL versus VAL. OR time was significantly longer in patients who underwent on-pump versus off-pump CABG, and OL versus VAL. The proportions of patients who developed deterioration in BI were higher in patients who underwent on-pump versus off-pump CABG, and OL versus VAL. The complication rate was higher in patients who underwent OL versus VAL (Table 2).

Age, sex, CCI, preoperative PCI, preoperative blood transfusion, hospital teaching status, hospital patient volume, and FY were associated with the choice of on-pump versus off-pump CABG; and sex, CCI, BI at admission, preoperative blood transfusion, preoperative blood purification, hospital teaching status, hospital patient volume, and FY were associated with the choice of OL versus VAL (Table 3).

Table 4 shows the intensive care procedures and outcomes in propensity score-matched CABG patients (n = 3,672) and lobectomy patients (n = 12,002). The rates of overall ICU stay, blood transfusion, and mechanical ventilation were higher in patients who underwent on-pump versus off-pump CABG, and VAL versus OL. OR time was significantly longer in patients who underwent on-pump versus off-pump CABG, and OL versus VAL. The complication rate was higher in patients who underwent OL versus VAL, but not in those who underwent on-pump versus off-pump CABG.

Complication rates were associated with the type of lung lobectomy but not the type of CABG (off-pump CABG: odds ratio 1.143 [95% confidence interval 0.973 to 1.344]; VAL: odds ratio 0.774 [95% confidence interval 0.688 to 0.870]). Off-pump CABG and VAL were not associated with deterioration in BI. In patients who underwent lobectomy, functional status at admission, mechanical ventilation, and blood transfusion were significantly associated with deterioration in BI, but complications were not (Table 5).

DISCUSSION

This study evaluated whether undergoing off-pump versus on-pump CABG, or VAL versus OL was associated with complication rates or functional outcomes. After adjustment for patient characteristics, preoperative critical care procedures and hospital characteristics, VAL was associated with a lower complication rate than OL. Complication rates and functional deterioration were not associated with on-pump versus off-pump CABG.

Previous studies evaluated the usefulness of off-pump CABG and VAL based on complication and mortality rates. Quality improvement initiatives in ICUs and multi-disciplinary cardiac rehabilitation programs have decreased mortality and complication rates and increased quality of life (Brahmbhatt et al., 2010; Suaya et al., 2009).

Table 1. Patient characteristics among patients receiving study surgical procedures (%) [SD].

Parameter	CABG			Lobectomy		P
	On-pump	Off-pump	P	Open	VAL	
Number	3901	3672		6029	14378	
Patient, hospital (academic, community)	128, 36	118, 34		189, 36	205, 37	
Age (Years)						
65-74	1568 (40.2)	1451 (39.5)		2319 (38.5)	5342 (37.2)	0.147
≥75	1012 (25.9)	1132 (30.8)	<0.001	1573 (26.1)	3906 (27.2)	
Mean, [SD]	67.7 (9.6)	68.9 (9.3)		67.0 (10.8)	67.3 (10.5)	0.058
Gender						
Male	2904 (74.4)	2887 (78.6)	<0.001	4186 (69.4)	8483 (59.0)	<0.001
Ambulance						
Use	526 (13.5)	425 (11.6)	0.012	22 (0.4)	32 (0.2)	0.071
Charlson comorbidity index						
1	1415 (36.3)	1317 (35.9)		1386 (23.0)	2753 (19.1)	
2	834 (21.4)	779 (21.2)	<0.001	871 (14.4)	2393 (16.6)	<0.001
3	295 (7.6)	333 (9.1)		422 (7.0)	1011 (7.0)	
4 or more	128 (3.3)	142 (3.9)		215 (3.6)	572 (4.0)	
BI at admission category						
Dependent	521 (13.4)	388 (10.6)	<0.001	47 (0.8)	133 (0.9)	0.183
Partially independent	128 (3.3)	117 (3.2)		70 (1.2)	133 (0.9)	
Hospital patient volume						
LVH	1396 (56.2)	1088 (43.8)		1942 (32.8)	3978 (67.2)	
MVH	1007 (44.1)	1275 (55.9)	<0.001	1938 (28.0)	4978 (72.0)	<0.001
HVH	1498 (53.4)	1309 (46.6)		2149 (28.4)	5422 (71.6)	
Teaching status						
Academic	692 (17.7)	1126 (30.7)	<0.001	1759 (29.2)	4975 (34.6)	<0.001
Fiscal year						
2006	980 (50.9)	945 (49.1)		1475 (34.8)	2758 (65.2)	
2007	601 (47.7)	659 (52.3)		1027 (35.5)	1862 (64.5)	
2008	643 (54.4)	540 (45.6)	0.007	1022 (34.0)	1982 (66.0)	<0.001
2009	692 (51.0)	665 (49.0)		1194 (29.9)	2802 (70.1)	
2010	985 (53.3)	863 (46.7)		1311 (20.9)	4974 (79.1)	

SD: Standard deviation. †: compared using analysis of variance. Other comparisons were made using chi-square test. CABG: coronary artery bypass graft, VAL: video assisted lobectomy, BI: barthel index, HVH: high volume hospital, LVH: low volume hospital, MVH: middle volume hospital.

2009). It is important to conduct studies of functional outcomes after MIS procedures to determine efficient allocation of healthcare financing and to improve outcomes in elderly patients (OECD StatExtracts, 2011; Suzuki, 2009).

In this study, the use of VAL was found to increase over the years, but the use of off-pump CABG was not. Some minimally invasive procedures may not have significant advantages over conventional procedures. For example, it was determined that the differences in outcomes after laparoscopic versus open appendectomy did not justify the increased costs of laparoscopic surgery, and the type of appendectomy performed is now determined by the preferences of patients and physicians (Cothren et al., 2005).

Table 2. Care process and outcomes among patients receiving study surgical procedures (%) [SD] (Cont).

Parameter	CABG			Lobectomy		
	On-pump	Off-pump	P	Open	VAL	P
PCI						
Overall	254 (6.5)	237 (6.5)	0.920			
Preoperative	125 (3.2)	118 (3.2)	0.982		***	
Postoperative	132 (3.4)	125 (3.4)	0.961			
ICU stay						
Overall	3375 (86.5)	2989 (81.4)	<0.001	2396 (39.7)	4080 (28.4)	<0.001
Preoperative	615 (15.8)	422 (11.5)	<0.001	16 (0.3)	16 (0.1)	0.011
Postoperative	3337 (85.5)	2958 (80.6)	<0.001	2392 (39.7)	4076 (28.3)	<0.001
Blood transfusion						
Overall	2890 (74.1)	2003 (54.5)	<0.001	663 (11.0)	508 (3.5)	<0.001
Preoperative	90 (2.3)	117 (3.2)	0.019	40 (0.7)	35 (0.2)	<0.001
Postoperative	2885 (74.0)	1996 (54.4)	<0.001	651 (10.8)	487 (3.4)	<0.001
Ventilation						
Overall	2767 (70.9)	2171 (59.1)	<0.001	226 (3.7)	217 (1.5)	<0.001
Preoperative	99 (2.5)	77 (2.1)	0.203	6 (0.1)	4 (0.0)	0.035
Postoperative	2751 (70.5)	2153 (58.6)	<0.001	223 (3.7)	215 (1.5)	<0.001
Cardiac support device						
Overall	740 (19.0)	637 (17.3)	0.067			
Preoperative	244 (6.3)	172 (4.7)	0.003		***	
Postoperative	681 (17.5)	604 (16.4)	0.243			
Blood purification						
Overall	394 (10.1)	355 (9.7)	0.529	38 (0.6)	123 (0.9)	0.097
Preoperative	273 (7.0)	274 (7.5)	0.436	30 (0.5)	106 (0.7)	0.055
Postoperative	386 (9.9)	345 (9.4)	0.462	38 (0.6)	120 (0.8)	0.129
Chemoradiation						
Chemotherapy				231 (3.8)	454 (3.2)	<0.001
Radiation		***		21 (0.3)	18 (0.1)	
Chemoradiation				23 (0.4)	16 (0.1)	
Operating room time (min)	475.5 [200.8]	409.1 [103.1]	<0.001†	328.4 [121.7]	280.5 [231]	<0.001†
Complications						
Minor complication	798 (20.5)	698 (19.0)	0.199	704 (11.7)	1652 (11.5)	<0.001
Major complication	16 (0.4)	11 (0.3)		31 (0.5)	21 (0.1)	
Abscess drainage	3 (0.1)	5 (0.1)	0.428	17 (0.3)	7 (0.0)	<0.001
Operative hemostasis	14 (0.4)	6 (0.2)	0.098	16 (0.3)	14 (0.1)	0.004
BI change						
Deterioration	235 (6.0)	203 (5.5)	<0.001	155 (2.6)	299 (2.1)	0.006

†: Compared using analysis of variance. Other comparisons were made using chi-square test. SD: standard deviation, BI: barthel index, CABG: coronary artery bypass graft, VAL: video assisted lobectomy, ICU: intensive care unit, PCI: percutaneous coronary intervention, ***: not examined.

Table 3. Factors associated with off-pump CABG and VAL.

Parameter	Off pump CABG	VAL
	Odds ratio (95% CI)	Odds ratio (95% CI)
Age (for 15 64 years)		
65-74 years	1.172 (1.049-1.309)	0.987 (0.919-1.060)
≥75 years	1.468 (1.298-1.660)	1.073 (0.991-1.161)
Gender		
Male	1.335 (1.194-1.493)	0.643 (0.603-0.687)
Ambulance		
Use	0.945 (0.803-1.113)	0.778 (0.435-1.391)
Charlson comorbidity index		
1	1.077 (0.961-1.207)	0.805 (0.744-0.871)
2	1.105 (0.967-1.262)	1.100 (1.005-1.204)
3	1.304 (1.080-1.575)	0.960 (0.848-1.087)
4 or more	1.373 (1.045-1.805)	1.055 (0.894-1.246)
BI at admission category (for nearly completely independent)		
Dependent	0.914 (0.772-1.082)	1.293 (0.909-1.840)
Partially independent	0.870 (0.668-1.134)	0.687 (0.509-0.929)
Preoperative PCI	1.368 (1.031-1.814)	-
Preoperative ICU stay	0.724 (0.606-0.866)	0.608 (0.283-1.307)
Preoperative Blood transfusion	1.615 (1.188-2.196)	0.412 (0.255-0.665)
Preoperative Ventilation	0.958 (0.683-1.344)	0.754 (0.177-3.204)
Preoperative Blood purification	0.973 (0.798-1.186)	1.529 (1.006-2.324)
Preoperative Cardiac support device	0.884 (0.691-1.130)	-
Teaching status (for community)		
Academic	2.048 (1.829-2.293)	1.180 (1.099-1.266)
Hospital patient volume		
MVH	1.492 (1.326-1.679)	1.241 (1.146-1.344)
HVH	0.975 (0.870-1.092)	1.198 (1.107-1.297)
Fiscal year		
2007	1.256 (1.085-1.453)	0.952 (0.861-1.052)
2008	0.931 (0.801-1.081)	1.018 (0.921-1.125)
2009	1.025 (0.888-1.182)	1.251 (1.139-1.374)
2010	0.869 (0.761-0.992)	2.024 (1.852-2.213)
Hosmer Lemeshow goodness of model fit		
p-value	0.266	0.026

CABG: Coronary artery bypass graft, VAL: video assisted lobectomy, CI: confidence interval, BI: barthel index, PCI: percutaneous coronary intervention, ICU: intensive care unit, HVH: high volume hospital. LVH: low volume hospital. MVH: middle volume hospital.

Researchers may well discover other advantages of MIS procedures that would justify our study (Angelini et al., 2009; Kapetanakis et al.,2008; Kiernan et al., 2011; Motallebzadeh et al., 2006; Rivera et al., 2011).

Administrative databases such as the one used in this study are useful for the evaluation of functional outcomes after MIS procedures. Studies in stroke patients found that functional status was low immediately after discharge

Table 4. Care process and outcomes among the propensity score matched patients receiving study surgical procedures (%) [SD].

Parameter	CABG			Lobectomy		
	On pump	Off pump	P	Open	VAL	P
Number of matched pairs	1836	1836		6001	6001	
PCI						
Overall	133 (7.2)	115 (6.3)	0.237			
Preoperative	67 (3.6)	54 (2.9)	0.229		***	
Postoperative	68 (3.7)	63 (3.4)	0.656			
ICU stay						
Overall	1580 (86.1)	1386 (75.5)	<0.001	2382 (39.5)	1551 (10.8)	<0.001
Preoperative	322 (17.5)	303 (16.5)	0.404	10 (0.2)	8 (0.1)	0.637
Postoperative	1561 (85.0)	1368 (74.5)	<0.001	2380 (39.5)	1550 (10.8)	<0.001
Blood transfusion						
Overall	1342 (73.1)	956 (52.1)	<0.001	643 (10.7)	214 (1.5)	<0.001
Preoperative	35 (1.9)	39 (2.1)	0.639	24 (0.4)	23 (0.2)	0.884
Postoperative	1338 (72.9)	953 (51.9)	<0.001	636 (10.5)	202 (1.4)	<0.001
Ventilation						
Overall	1275 (69.4)	1077 (58.7)	<0.001	217 (3.6)	97 (0.7)	<0.001
Preoperative	53 (2.9)	43 (2.3)	0.301	1 (0.0)	2 (0.0)	0.564
Postoperative	1264 (68.8)	1068 (58.2)	<0.001	216 (3.6)	96 (0.7)	<0.001
Cardiac support device						
Overall	362 (19.7)	372 (20.3)	0.680			
Preoperative	132 (7.2)	115 (6.3)	0.263		***	
Postoperative	330 (18.0)	356 (19.4)	0.271			
Blood purification						
Overall	178 (9.7)	159 (8.7)	0.277	38 (0.6)	41 (0.3)	0.735
Preoperative	127 (6.9)	120 (6.5)	0.645	30 (0.5)	32 (0.2)	0.799
Postoperative	173 (9.4)	153 (8.3)	0.246	38 (0.6)	41 (0.3)	0.735
Chemoradiation						
Chemotherapy				228 (3.8)	233 (1.6)	0.002
Radiation		***		21 (0.3)	4 (0.0)	
Chemoradiation				23 (0.4)	12 (0.1)	
Operating room time (min)	467.3 [256.2]	406.4 [98.1]	<0.001	328.4 [121.5]	288.8 [332.4]	<0.001
Complications						
Minor complication	373 (20.3)	412 (22.4)	0.214	702 (11.6)	597 (4.2)	<0.001
Major complication	8 (0.4)	5 (0.3)		31 (0.5)	7 (0.0)	
Abscess drainage	3 (0.1)	3 (0.1)	1.000	17 (0.3)	1 (0.0)	<0.001
Operative hemostasis	6 (0.3)	2 (0.1)	0.157	16 (0.3)	6 (0.0)	0.033
BI change category						
Deterioration	101 (5.5)	92 (5.0)	0.207	150 (2.5)	127 (0.9)	0.374

†: Compared using analysis of variance. Other comparisons were made using chi-square test. SD: standard deviation, CABG: coronary artery bypass graft, VAL: video assisted lobectomy, BI: barthel index, PCI: percutaneous coronary intervention. ***: not examined.

Table 5. Factors associated with complications and BI deterioration.

Parameter	Complications		BI deterioration	
	CABG	Lobectomy	CABG	Lobectomy
	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Age (for 15-64 years)				
65-74 years	0.889 (0.737-1.073)	1.058 (0.919-1.217)	1.676 (1.090-2.576)	1.863 (1.285-2.700)
≥75 years	0.872 (0.697-1.091)	1.173 (1.010-1.362)	4.505 (2.901-6.996)	4.230 (2.976-6.014)
Gender				
Male	0.920 (0.763-1.110)	0.951 (0.837-1.079)	0.957 (0.683-1.342)	0.951 (0.725-1.248)
Ambulance				
Use	1.218 (0.928-1.598)	0.878 (0.254-3.036)	0.982 (0.585-1.648)	0.670 (0.079-5.694)
Charlson comorbidity index				
1	1.506 (1.228-1.846)	1.248 (1.08-1.442)	0.781 (0.532-1.147)	1.373 (1.015-1.856)
2	1.665 (1.326-2.092)	1.264 (1.065-1.500)	1.052 (0.696-1.59)	1.190 (0.820-1.727)
3	1.225 (0.845-1.777)	0.978 (0.770-1.242)	0.781 (0.380-1.604)	1.656 (1.086-2.526)
4 or more	1.543 (0.930-2.561)	1.671 (1.277-2.188)	1.486 (0.720-3.067)	1.562 (0.886-2.754)
BI at admission category (for nearly completely independent)				
Dependent	1.006 (0.766-1.321)	1.114 (0.556-2.233)	0.320 (0.175-0.586)	3.511 (1.571-7.846)
Partially independent	1.178 (0.773-1.794)	0.979 (0.576-1.665)	1.023 (0.521-2.007)	4.543 (2.607-7.918)
PCI†	1.183 (0.732-1.911)	***	0.639 (0.301-1.357)	***
ICU stay†	1.041 (0.783-1.384)	1.559 (0.431-5.637)	1.146 (0.741-1.774)	1.199 (0.924-1.555)
Blood transfusion†	0.740 (0.371-1.474)	1.028 (0.422-2.502)	1.329 (0.892-1.981)	1.958 (1.368-2.803)
Ventilation†	0.690 (0.380-1.255)	-	1.256 (0.887-1.779)	3.331 (2.120-5.234)
Blood purification†	0.730 (0.503-1.059)	1.870 (0.959-3.649)	3.079 (1.973-4.803)	2.981 (1.290-6.888)
Cardiac support device†	0.646 (0.432-0.967)	***	1.162 (0.782-1.725)	***
Chemotherapy or radiation				
Chemotherapy		0.868 (0.615-1.227)		1.381 (0.771-2.475)
Radiation	***	0.844 (0.191-3.728)	***	3.247 (0.684-15.419)
Chemoradiation		0.296 (0.040-2.212)		**

Table 5. Contd.

Use of innovation				
Off pump or VAL	1.143 (0.973-1.344)	0.774 (0.688-0.870)	1.049 (0.769-1.432)	0.922 (0.717-1.187)
Complication	***	***	1.306 (0.921-1.851)	0.559 (0.365-0.857)
Teaching status (for community)				
Academic	0.984 (0.689-1.407)	0.720 (0.623-0.832)	0.706 (0.344-1.447)	1.133 (0.842-1.523)
Hospital patient volume (for LVH)				
MVH	1.016 (0.795-1.298)	1.593 (1.365-1.860)	1.303 (0.827-2.052)	1.168 (0.852-1.602)
HVH	1.288 (1.079-1.537)	1.146 (0.982-1.338)	1.388 (0.977-1.972)	0.701 (0.500-0.981)
Fiscal year (for 2006)				
2007	1.256 (0.937-1.685)	1.265 (0.999-1.602)	0.731 (0.423-1.265)	1.341 (0.854-2.106)
2008	1.955 (1.499-2.550)	1.625 (1.300-2.032)	0.532 (0.310-0.913)	1.380 (0.877-2.172)
2009	2.238 (1.726-2.903)	2.039 (1.655-2.511)	0.776 (0.474-1.270)	1.721 (1.142-2.592)
2010	2.201 (1.723-2.811)	5.014 (4.152-6.055)	1.328 (0.890-1.981)	2.557 (1.747-3.742)
Operating room time				
One more minute longer	1.0001 (0.9998-1.0005)	1.000 (0.9999-1.0003)	1.0004 (0.9999-1.0008)	1.000 (0.9997-1.0004)
Hosmer Lemeshow goodness of model fit				
P-value	0.094	0.311	0.587	0.577

CABG: coronary artery bypass graft. VAL: video assisted lobectomy, CI: confidence interval, BI: barthel index, PCI: percutaneous coronary intervention, ICU: intensive care unit, HVH: high volume hospital, MVH: middle volume hospital, LVH: low volume hospital, ***: originally not included, **: no cases with BI deterioration and chemo-radiation, †: preoperative critical care use in the complication model and overall use during the hospitalization in the BI deterioration model.

and started to recover after about 30 days, reaching a plateau at 90 to 180 days (Dowdy et al., 2005; Sacanella et al., 2011; Sulter et al., 1999). In this study, the mean lengths of stay were 32 or 37 days for CABG and 15 or 22 days for lobectomy, which is longer than the lengths of stay reported in Western countries (Gopaldas et al., 2010; Kiernan et al., 2011; Mishra et al., 2006; Rivera et al., 2011). The OECD has acknowledged that acute care hospitals have different roles in Japan than in Western countries (OECD, 2005). The longer length of stay in Japanese hospitals gives us additional information regarding postoperative functional outcomes. In this study, functional outcomes were not significantly better after off-pump versus on-pump CABG, which is consistent with the results of other studies that measured quality of life using the Short-Form 36, and found no significant differences in general health status at 30 to 360 days between patients who underwent on-pump versus off-pump CABG

(Angelini et al., 2009; Kapetanakis et al., 2008; Motallebzadeh et al., 2006). Functional deterioration was not significantly different after VAL versus OL, although the complication rate was lower after VAL. This finding could provide further validation of the usefulness of VAL, in combination with the findings of other studies that evaluated the advantages and disadvantages of VAL versus OL (Kiernan et al., 2011).

As lower BI at admission and advancing FY were associated with higher complication rates and worse functional outcomes in patients who underwent lobectomy, policymakers may pay more attention to promoting VAL than off-pump CABG. Patients who underwent lobectomy may have had different preoperative characteristics than those who underwent CABG. Unlike CABG, lobectomy inherently reduces vital organ capacity, and functional recovery is therefore expected to be less after lobectomy than after CABG. As pre- and postoperative, some critical care procedures were associated with BI deterioration in BI for lobectomy patients; it is more important to monitor the quality of critical care rather than in CABG patients. As functional status at admission was associated with functional outcomes in this study, as well as in other studies, appropriate indications for lobectomy should be determined, and relevant skills training and coordination of multidisciplinary treatment among intensivists, cardiologists, OR staff, and rehabilitation staff should be encouraged (Brahmbhatt et al., 2010; Holzhey et al., 2008; Suaya, 2009).

This study has some limitations that should be considered. First, only data from patients discharged during a 6-month period each year for 5 years were analyzed. Even though patients were matched for many significant covariates that could affect the choice of MIS versus conventional procedures, there may be additional variables associated with this choice that were not taken into consideration. The database has now started to record the postal codes of patients, and the study period has been extended to include the whole year from 2010. The methodology of future observational studies can therefore be strengthened by analyzing the distance between home and hospital and by using a larger database (Suaya et al., 2009).

Second, some variables such as the American Society of Anesthesiologists score and cancer stage were not included in the analyses. As there are concerns that these scales do give precise indications of functional status, the current study analyzed intensive care procedures, indicating organ failure and advanced cancer stage instead. For example, use of intra-aortic balloon pumping indicates severe coronary artery disease, and administration of chemoradiation therapy indicates advanced cancer stage. These analyses also support the development of quality improvement initiatives for critical care procedures (Brahmbhatt et al., 2010).

Finally, patients aged less than 15 years or with missing BI data were excluded from our analyses. Although the differences between the included and excluded patient groups appear to be significant, these differences are not large enough to distort our results (Annex Table 1).

Conclusion

This study evaluated differences in complication rates and functional outcomes between patients who underwent on-pump versus off-pump CABG, and OL versus VAL. In propensity score-matched groups of patients who underwent CABG and lobectomy, postoperative complication rates were lower in patients who underwent VAL versus OL, but not off-pump versus on-pump CABG. Functional deterioration was not significantly different between MIS and conventional procedures for either CABG or lobectomy. As the physical status at admission was associated with functional outcome in patients who underwent lobectomy, the appropriate indications for VAL should be determined, and perioperative treatment strategies to maintain functional status during hospitalization should be implemented.

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Annex Table 1. Comparison of patient characteristics, care process and outcomes between the excluded and included population (%) [SD].

Parameter	CABG			Lung lobectomy		
	Excluded group	Included group	P	Excluded group	Included group	P
Patient number (academic, community)	13523	7573		33490	20407	
Hospital number (academic, community)	78, 302	36, 131		81, 609	37, 218	
Innovation						
Off pump or VAL	7093 (52.5)	3672 (48.5)		22168 (66.2)	14378 (70.5)	<0.001
Mean, [SD]	68.2 [9.5]	68.3 [9.5]	0.329†	66.7 [11.4]	67.2 [10.6]	<0.001†
Age						
Under 15 years	7 (0.1)	0 (0.0)		186 (0.6)	0 (0.0)	
15-64 years	4365 (32.3)	2410 (31.8)	0.188	12052 (36.0)	7267 (35.6)	<0.001
65-74 years	5399 (39.9)	3019 (39.9)		12560 (37.5)	7661 (37.5)	
≥75 years	3752 (27.7)	2144 (28.3)		8692 (26.0)	5479 (26.8)	
Gender						
Male	10299 (76.2)	5791 (76.5)	0.612	20555 (61.4)	12669 (62.1)	0.102
Ambulance						
Used	2084 (15.4)	951 (12.6)	<0.001	124 (0.4)	54 (0.3)	0.038
Charlson comorbidity index						
1	4695 (34.7)	2732 (36.1)		6308 (18.8)	4139 (20.3)	
2	3035 (22.4)	1613 (21.3)	0.164	5192 (15.5)	3264 (16.0)	<0.001
3	1080 (8.0)	628 (8.3)		2156 (6.4)	1433 (7.0)	
4 or more	476 (3.5)	270 (3.6)		1315 (3.9)	787 (3.9)	
Teaching status						
Academic	4241 (31.4)	1818 (24)	<0.001	10411 (31.1)	6734 (33.0)	<0.001
Fiscal year						
2006	1537 (44.4)	1925 (55.6)		2890 (40.6)	4233 (59.4)	
2007	3517 (73.6)	1260 (26.4)		8472 (74.6)	2889 (25.4)	
2008	3496 (74.7)	1183 (25.3)	<0.001	8140 (73.0)	3004 (27.0)	<0.001
2009	2111 (60.9)	1357 (39.1)		5685 (58.7)	3996 (41.3)	
2010	2862 (60.8)	1848 (39.2)		8303 (56.9)	6285 (43.1)	
PCI						
Overall	929 (6.9)	491 (6.5)	0.283		***	
Preoperative	499 (3.7)	243 (3.2)	0.069			
Postoperative	463 (3.4)	257 (3.4)	0.908			

Annex Table 1. Contd.

Parameter	CABG			Lung lobectomy		
	Excluded group	Included group	P	Excluded group	Included group	P
ICU stay						
Overall	11538 (85.3)	6364 (84)	0.012	11788 (35.2)	6476 (31.7)	<0.001
Preoperative	1975 (14.6)	1037 (13.7)	0.07	75 (0.2)	32 (0.2)	0.089
Postoperative	11373 (84.1)	6295 (83.1)	0.065	11756 (35.1)	6468 (31.7)	<0.001
Blood transfusion						
Overall	8664 (64.1)	4893 (64.6)	0.430	2001 (6)	1171 (5.7)	0.257
Preoperative	468 (3.5)	207 (2.7)	0.004	151 (0.5)	75 (0.4)	0.146
Postoperative	8634 (63.8)	4881 (64.5)	0.379	1922 (5.7)	1138 (5.6)	0.429
Ventilation						
Overall	9281 (68.6)	4938 (65.2)	<0.001	834 (2.5)	443 (2.2)	0.018
Preoperative	382 (2.8)	176 (2.3)	0.030	41 (0.1)	10 (0.0)	0.007
Postoperative	9227 (68.2)	4904 (64.8)	<0.001	813 (2.4)	438 (2.1)	0.035
Cardiac support device						
Overall	2740 (20.3)	1377 (18.2)	<0.001			
Preoperative	949 (7.0)	416 (5.5)	<0.001		***	
Postoperative	2508 (18.5)	1285 (17.0)	0.004			
Blood purification						
Overall	1467 (10.8)	749 (9.9)	0.030	241 (0.7)	161 (0.8)	0.364
Preoperative	1027 (7.6)	547 (7.2)	0.325	212 (0.6)	136 (0.7)	0.639
Postoperative	1428 (10.6)	731 (9.7)	0.037	238 (0.7)	158 (0.8)	0.402
Chemoradiation						
Chemotherapy				1426 (4.3)	685 (3.4)	
Radiation		***		80 (0.2)	39 (0.2)	<0.001
Chemoradiation				91 (0.3)	39 (0.2)	
Operating room time (minute)	435.7 [273.4]	443.3 [164.4]	0.029†	285.3 [112.5]	294.6 [206]	0.772†
Complication						
Minor complication	2423 (17.9)	1496 (19.8)	0.004	4378 (13.1)	2356 (11.5)	<0.001
Major complication	49 (0.4)	27 (0.4)	-	92 (0.3)	52 (0.3)	-
Abscess drainage	17 (0.1)	8 (0.1)	0.684	45 (0.1)	24 (0.1)	0.598
Operative hemostasis	32 (0.2)	20 (0.3)	0.700	50 (0.1)	30 (0.1)	0.947

CABG: coronary artery bypass graft, VAL: video assisted lobectomy, SD: standard deviation, †: compared using analysis of variance. Other comparisons were made using chi-square test. BI: barthel index, ICU: intensive care unit, PCI: percutaneous coronary intervention, ***: not examine.

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Full Length Research Paper

In vitro preventive effects of nitrate tolerance by a polyphenol-enriched extract of *Hibiscus sabdariffa*

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Treatment failure or tolerance, which rapidly leads to a reduced hemodynamic effects and therapeutic efficacy is the major limitation of long-term use of nitrates, including nitroglycerin (NTG) in the treatment of coronary artery disease. These effects are most often associated with oxidative stress. Thus, in this work, we were interested in the prevention of nitrate tolerance by the antioxidant compounds from *Hibiscus sabdariffa* L. crude extract, a plant from the Senegalese Pharmacopoeia, rich in polyphenols. Thoracic aorta segments without endothelium were taken from rats and incubated in isolated organ chambers. The vessels were then pre-exposed with the *H. sabdariffa* polyphenolic extract (HSE, 5.10^{-2} g/l) or antioxidants such as N-acetyl cysteine (NAC, 10^{-3} M) or vitamin C (VIT C, 10^{-2} M), taken as reference. After a 30 min treatment, aortic segments were exposed to NTG (50 µM, 1 h) to induce tolerance state before being contracted to adrenaline (10^{-8} to 10^{-5} M), and then relaxed with NTG (10^{-9} to 10^{-5} M). Polyphenols from *H. sabdariffa* potentiated the relaxant response to NTG, whatever the state of vascular tolerance; the HSE partially corrected the *in vitro* nitrate tolerance. This work suggests interesting therapeutic perspectives by improving the response to treatment with nitrates in coronary patients.

Key words: Nitrate tolerance, antioxidant, vascular diseases, therapeutic agents, medicinal plants.

INTRODUCTION

Angina pectoris, usually due to coronary heart disease, is recognized as a transient retrosternal pain syndrome and characteristic of myocardial ischemia. It occurs more readily after 50 years and more frequently favored by risk factors: hypertension, diabetes, dyslipidemia, smoking, obesity, heredity (Stritzke et al., 2009). Prevalence is difficult to quantify and varies by country. However, it is higher in industrialized countries where it affects about 2% of the population (Carevic et al., 2007). In the

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treatment of myocardial infarction, the first-line drugs consist of nitrates, including nitroglycerin. However, their long-term use is limited by a therapeutic escape or tolerance effect, which decreases the therapeutic efficacy of the drug, compromising the patient's prognosis (Munzel et al., 2005; Daiber et al., 2010a; b; Munzel, 2008).

In the mechanisms of tolerance to nitrates, two major phenomena are discussed:

(i) a pseudotolerance phenomenon (Munzel et al., 2005), linked to a neurohormonal activity, associated with volume expansion, both intended to limit the fall in blood pressure associated with a vasodilatory nitrates effect, and; (ii) a direct loss of vascular response called vascular tolerance (Wenzl et al., 2009; Fink and Bassenge, 2002; Wang et al., 2002b), whose mechanism is not fully elucidated. In the current state of knowledge, oxidative stress is a major cause of this phenomenon (Fadel et al., 2012; Oelze et al., 2010; Daiber et al., 2004; 2005; Mollnau et al., 2006).

Thus, our hypothesis derives from the antioxidant capacity of compounds to reduce or prevent vascular tolerance to nitrates, by reducing oxidative stress.

Antioxidant compounds have shown a growing interest to researchers. Indeed, studies have demonstrated their ability to prevent nitrate tolerance in animal models, including humans. These agents include natural antioxidants such as vitamin C, vitamin E and plant polyphenols. However, studies have shown that vitamins are able to produce free radicals with cytotoxic effects (Satoh et al., 1996; Kagan et al., 1994; Cai and Harrison, 2000), unlike the polyphenols. They are abundantly synthesized by plants, hence our interest in *H. sabdariffa* L., a plant from the Senegalese Pharmacopoeia, rich in polyphenols. Therefore, the objective of this work was to study the experimental conditions for the prevention of tolerance to nitroglycerin, and specifically demonstrate the *in vitro* antioxydant and preventive effects of *H. sabdariffa*.

MATERIALS AND METHODS

Drugs

Norepinephrine and Acetylcholine were purchased from Sigma Chemical Co; N-acetyl cysteine (Fluimucil® 5 g/25 ml, solution for infusion) and Vitamine C (VITAMIN C 10 Percent, AGUETTANT®, solution for injection, for infusion) was purchased from a local drugstore. Nitroglycerin (0.15 mg, Sublingual Tablets) was provided to us by the hospital pharmacy (Hôpital Principal) in Dakar (Senegal). All drugs were serially diluted in distilled water before each experiment. The concentration of the drugs is expressed as final molar concentration in the bath.

Hibuscus sabdariffa extract preparation

H. sabdariffa calyces was obtained from botanical laboratory of our faculty and prepared as previously described (Sarr et al., 2009). In brief, calyces were dried during a week at room temperature. Dried and powdered calyx (Grinder RM-100, Retsch®) of *H. sabdariffa* (500 g) was extracted by maceration at room temperature for 2 h with 60% methanol. The hydroalcoholic extract was then filtered in vacuum conditions (Vacuum pump V-700, Büchi®) by means of the phial of Kitassato and evaporated on a rotary evaporator (Rotavapor R-210, Büchi®). Methanolic extract evaporation was realized during three successive days until the obtaining of a dry crude extract.

Organ chambers experiments

Vessels preparation

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as promulgated by the Senegalese Academic Bioethics Committee. Male Wistar rats weighing 150 to 200 g were procured from a local Institute. They were fed on standard rat feed and given free access to water. After anesthesia by intraperitoneal injection of pentothal (60 mg/kg body weight) for 10 min, rats were sacrificed and exsanguinated by cross section of the carotid. After supra-umbilical laparotomy, the thoracic aorta was removed carefully from the bottom up, transferred into a petri dish filled with Krebs solution of the following composition (in mM): (NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25 and D-glucose 11, pH 7.4, 37°C) and cleaned of adherent connective tissue. As indicated, the endothelium was removed by rubbing the intimal surface of rings with a pair of forceps and cut into 3 mm ring segments.

Protocol design

Dose response studies are typically conducted to assess concentration-response relationships in isolated rat thoracic aorta preparation which allow maintaining the integrity of the tissue for several hours in a temperature-controlled environment, while physiological measurements are performed. The rings were suspended between two wire hooks in organ chambers filled with 10 ml of Krebs solution (37°C, pH 7.40) aerated with O_2 95%/CO_2 5%. The upper hook was connected to a force transducer (Panlab-TRI 202P), and changes in isometric force were recorded (Labscribe® Iworx/118 Data Recording Sofware). After a resting tension (1 g) defined by preliminary studies, the rings were allowed to equilibrate for 45 min and were then precontracted with 60 mM potassium chloride to determine maximal contraction. The rings were washed twice with a fresh buffer solution and then pre-contracted with norepinephrine (10^{-6} M) to reach an optimal constriction (80% maximum). Pre-contracted rings were allowed to plateau and then acetylcholine (10^{-6} M) was added to assess the endothelial function. Only rings that exhibited less than 10% relaxation responses to acetylcholine were considered without endothelium and used in subsequent experiments. Rat aortic segments were then randomly assigned to one of the 8 groups: 1 - Control; 2 - Tolerant (Nitroglycerin (NTG)-treated); 3 - N-acétyl cysteine (NAC) ; 4 - Vitamin C (VIT C) ; 5 - *H. sabdariffa* extract (HSE), 6 - VIT C-tolerant; 7 - NAC-tolerant and 8 - HSE-tolerant. The concentration of HSE chosen (5.10^{-2} mg/ml) was based on concentration-response data obtained in a previous study (Sarr et al., 2009).

Nitrate tolerance induction

To induce nitrate tolerance, thoracic aortic segments without

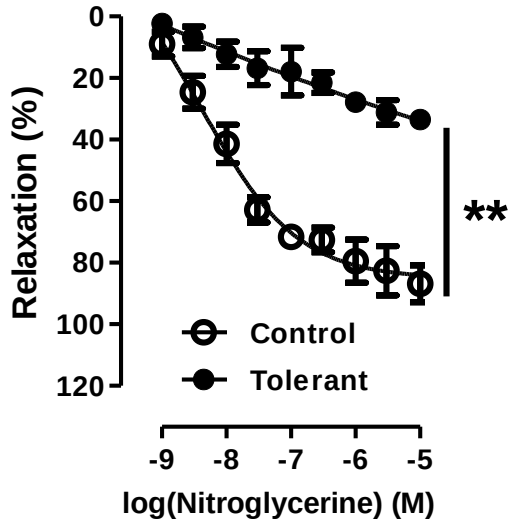

Figure 1. Concentration-effect curves of relaxant response to nitroglycerin in rat thoracic aorta without endothelium (Control) or pre-exposed (Tolerant) to nitroglycerin (NTG, 5.10^{-5} M, 60min). Results are presented as mean ± SEM of 7 experiments from 7 rats different. ** = P < 0.01 (2-way ANOVA).

Table 1. Relaxing responses of nitroglycerin expressed in percent and EC_{50} values determined by log-logit regression in rat thoracic aorta without endothelium or tolerant vessels pre-exposed to *H. sabdariffa* extract (HSE), N-acetylcysteine (NAC) or Vitamin C (VIT C).

Parameter	EC_{50} (M)	E_{max} (%)
Control	$1.01\times10^{-8}\pm0.05$	81.16±7.42
Tolerant	$9.69\times10^{-8}\pm0.83$	31.33±4.23
HSE	$4.68\times10^{-9}\pm0.5$	111±13.91
NAC	$1.16\times10^{-7}\pm0.03$	119±18.74
VIT C	$1.62\times10^{-8}\pm0.09$	94.19±11.32
HSE-tolerant	$4.75\times10^{-8}\pm0.64$	66.71±7.54
NAC-tolerant	$3.53\times10^{-8}\pm0.08$	61.73±9.26
VIT C-tolerant	$1.54\times10^{-7}\pm0.12$	96.40±21.13

endothelium were first exposed to NTG (50 µM, 1 h) and were pre-contracted with cumulative concentrations of norepinephrine (10^{-8} to 10^{-5} M). When a plateau phase was obtained, cumulative concentration-response curves were obtained with NTG concentrations ranging from 10^{-9} to 10^{-5} M.

Prevention of nitroglycerin-induced nitrate tolerance

Before induction of tolerance state, the aortic segments were pre-exposed to one of the antioxidants taken as reference. To do this, they were treated with NAC (10^{-3} M), VIT C (10^{-2} M) or HSE (0.1 mg/ml). After 30 min exposition, they were pre-contracted with norepinephrine (10^{-8} to 10^{-5} M). When a plateau phase was reached, cumulative concentration-response curves were obtained with NTG concentrations ranging from 10^{-9} to 10^{-5} M.

Statistical analysis

Values are expressed as mean ± standard error of mean (SEM). Concentration-response curves were compared by 2-way Analysis of Variance (ANOVA) or the multi-analysis of variance (MANOVA), as required. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Nitrate tolerance induction

Results obtained in our study (Figure 1) clearly indicate a tolerance state in NTG-treated vessels. Indeed, after exposure of aorta rings with NTG, there was a significant reduction of at least 40% of the maximum relaxation to nitroglycerin (tolerant vessels: half maximal effective concentration (EC_{50}) = $9.695 \times 10^{-8} \pm 0.831$ M; E_{max} = 31.33 ± 4.23%) compared to control (non-tolerant vessels: $EC_{50} = 1.015 \times 10^{-8} \pm 0.054$ M; E_{max} = 81.16 % ± 7.42) as indicated in Table 1. This loss of the ability of relaxation observed in tolerant vessels highlights, in our model, the phenomenon of treatment failure associated with prolonged use of nitrates.

Potentiating effects of *H. sabdariffa* extract on the relaxant responses to nitroglycerin

In order to examine any potentiating effect on the NTG-induced relaxations (10^{-9} to 10^{-5} M) after HSE pre-exposure at a final concentration of 5.10^{-2} mg/ml during 30 min, we compare relaxations in HSE-exposed vessels with those obtained with control (not pre-exposed). Results obtained in Figure 2A showed in HSE-exposed vessels, a potentiating of the relaxant response to NTG ($EC_{50} = 4.679 \times 10^{-9} \pm 0.498$ M; E_{max} = 111% ± 13.91) compared to control vessels, for which the maximal effect is only about 81.16% ± 7.42 ($EC_{50} = 1.015 \times 10^{-8} \pm 0.054$ M). This demonstrates the ability of the *H. sabdariffa* polyphenolic extract to potentiate the pharmacological vasorelaxant effects of nitrates (Table 1).

In parallel, we studied the effects of two known anti-oxidants, namely NAC and VIT C on the relaxant responses to NTG in order to verify whether the potentiating effects of HSE can be due to its antioxidant character. For this, we also compare relaxations obtained after NAC or VIT C pre-exposure with those obtained with control not exposed. Our results as indicated in Figure 2B actually showed a potentiating effect of NAC only at high concentrations (10^{-5} M NTG) compared to the control vessels (EC_{50}: $1.015 \times 10^{-8} \pm 0.054$ M versus 1.163×10^{-7}

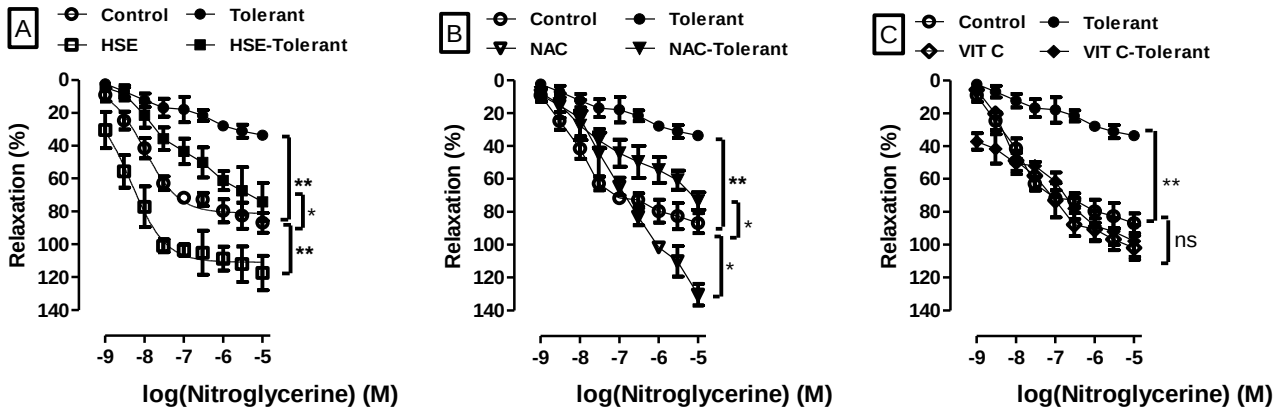

Figure 2. Concentration-effect curves of relaxant responses to nitroglycerin in rat thoracic aorta without endothelium (Control) or tolerant vessels (NTG, 5.10^{-5} M, 60 min), pre-exposed to (A) *H. sabdariffa* extract (HSE, 5.10^{-2} mg/ml), (B) N-acetylcysteine (NAC, 10^{-3} M) and (C) Vitamin C (VIT C, 10^{-2} M) during 30 min.
Results are presented as mean ± SEM of 7 experiments from 7 different rats. ns = not significant, * = $p < 0.05$, ** = $p < 0.01$ (MANOVA) compared to control.

± 0.029 and E_{max}: 81.16% ± 7.42 versus 119% ± 18.74; Control versus NAC, respectively). For Vit C, as indicated on Figure 2C, no significant improvements in relaxation responses were observed (EC_{50}: 1.015 ± 0.054 × 10^{-8} M versus 1.620 ± 0.091 × 10^{-8} and E_{max}: 81.16% ± 7.42 versus 94.19% ± 11.32; Control versus VIT C, respectively). Thus, compared to NAC, the HSE better potentiates the vasorelaxant response to NTG, since this potentiating was observed for all doses in the range of relaxation. These results indicate a potential benefit role of HSE, while more potentiating the relaxant responses of NTG compared to other antioxidants NAC and VIT C.

Normalizing effects of *H. Sabdariffa* extract in tolerance conditions

Since some antioxidants potentiate the relaxant responses of NTG, we first sought to determine whether the responses in antioxidant-tolerant aortic segments were different to those obtained in control vessels (without any treatment). If it is the case, we can conclude a normalizing effect by the antioxidant. The results, as shown in Figure 2A, show a significant difference between the HSE-tolerant (EC_{50} = 4.75 × 10^{-8} ± 0.64; E_{max} = 66.71% ± 7.54) and control vessels (EC_{50}: 1.015 × 10^{-8} ± 0.054 M; E_{max}: 81.16% ± 7.42) demonstrating the non possibility of HSE to normalize the relaxant responses to NTG in tolerant vessels. Also, in comparison to the same untreated control, NAC-treated tolerant vessels did not normalize the relaxing effects of cumulatively administered NTG (Figure 2B), since a significant difference was observed between NAC-tolerant (EC_{50} = 3.53 × 10^{-8} ± 0.08 M; E_{max} = 61.73% ± 9.26) and control vessels (EC_{50}: 1.015 × 10^{-8} ± 0.054 M; E_{max}: 81.16% ± 7.42).

However, this result contrasts with those obtained with VIT C, under the same conditions. Indeed, VIT C, without having potentiating effects on NTG-induced relaxation, normalized relaxation responses. Indeed, the results in Figure 2C showed no significant difference between VIT C-tolerant vessels (EC_{50}= 1.62 ± 0.09.10^{-8} M; E_{max} = 94.19 % ± 11.32) and non-tolerant controls (EC_{50}: 1.015 ± 0.054 × 10^{-8} M; E_{max}: 81.16% ± 7.42). It is interesting to note that VIT C has only potentiating effect in tolerant vessels.

Preventive role of *H. Sabdariffa* extract on the tolerance induction

In order to investigate the ability of HSE to prevent the development of tolerance, we first incubated aortic segments with the HSE before inducing the tolerance phenomenon. A preventive effect on tolerance induction was obtained when any significant difference was observed between relaxant responses in HSE-treated vessels with those of HSE-tolerant vessels. Figure 2A showed a significant difference in the relaxant responses between HSE (EC_{50} = 4.68 × 10^{-9} ± 0.5 M; E_{max} = 111% ± 13.91) and HSE-tolerant vessels (EC_{50} = 4.75 × 10^{-8} ± 0.64; E_{max} = 66.71% ± 7.54), showing a continuing state of tolerance. Thus, we conclude that HSE is not able to prevent nitrates tolerance, as well as NAC (Figure 2B). By contrast, VIT C completely reverse tolerance to nitroglycerin and any significant different were found between VIT-treated and VIT-tolerant vessels as shown in Figure 2C.

DISCUSSION

The aim of the present study was to evaluate the ability of a Senegalese and African Pharmacopoeia plant extract to prevent the development of nitrates tolerance. In light of all of our results, the polyphenol-rich extract of *H. sabdariffa* are able to potentiate the relaxant responses to nitroglycerin in non-tolerant and tolerant denuded-thoracic aortic rings; partially correcting the nitrate tolerance by normalizing the relaxant responses to nitroglycerin but can not prevent the development of this phenomenon.

Regarding the methodological approach, we used an *in vitro* nitrates tolerance induction model, which was already validated in numerous studies (Wang et al., 2002a; Van de Voorde et al., 1987, 1994; Stewart et al., 1989; Ratz et al., 2000; Otto et al., 2005; Chegaev et al., 2009; Bennett et al., 1988; Sarr et al., 2005). To do this, we used aortic denuded-rings in order to avoid possible interference between the exogenous nitrogen oxide (NO) from nitroglycerin and endothelium-derived one. The model is established when we observe a significant reduction in the relaxant response to nitroglycerin. This simple model allows overcoming the influence of neurohumoral counter-regulatory involved in vascular physiology.

Among factors involved in reducing the bioavailability of NO, recent work reported by researchers confirm that the development of tolerance to nitrates is associated with increased oxidative stress (Oelze et al., 2010; Daiber et al., 2009a; DiFabio et al., 2006; Muller et al., 2004; Parker, 2004; Gori et al., 2001; Laight et al., 1997, 1998). In addition, a key enzyme involvement of nitroglycerin and nitrates biotransformation, namely the mitochondrial aldehyde dehydrogenase (ALDH 2), is inhibited by reactive oxygen species (ROS) which in addition, can degrade nitric oxide (Wenzel et al., 2009a, 2009b; Daiber et al., 2004, 2009b; Sydow et al., 2004). Therefore, it would be interesting to study the benefit of antioxidants in preventing nitrate tolerance development.

Regarding the natural antioxidants, various therapeutic interventions have been considered and evaluated experimentally or clinically in order to improve endothelial function, the evolution of certain diseases affecting the vascular system and the prevention of tolerance to nitrates. These natural antioxidants include vitamin C, vitamin E and flavonoids. Moreover, the results of many clinical studies, particularly the Heart Outcomes Prevention Evaluation (HOPE) study (Yusuf et al., 2000) showed that vitamin E had no beneficial effect in patients with high cardiovascular risk. The Italian Group for the Study of Streptokinase nell'Infarto (GISSI) study (Hopper et al., 1999) meanwhile showed that in contrast to polyunsaturated fatty acids n-3 (or omega 3), vitamin E does not decrease significantly the incidence of cardiovascular events among patients who have survived a myocardial infarction. These studies have also reported the limited effectiveness of these antioxidant vitamins, especially their low bioavailability following oral administration or some compartmentalization problems, and their ability to produce free radicals after reacting with oxidizing molecules. Thus it has been reported that vitamin E is capable of producing a tocopheroxyl radical and in the case of vitamin C, ascorbyl radical can be generated (Carr and Frei, 2000), to the extent that until now the results obtained with different classes of antioxidants, including vitamins, are not really satisfactory and further studies with other compounds remains justified.

Indeed, it is now accepted that polyphenols could prevent many diseases such as cancers (Yang et al., 2009; Korkina et al., 2009; Khan et al., 2009; Bracke et al., 2008; Franklin and McCubrey, 2007), cardiovascular (Madeira et al., 2009; Jones et al., 2011; Kumar et al., 2008; Agouni et al., 2009; Walter et al., 2008; Schini-Kerth et al., 2011; Sall Diallo et al., 2008; Sarr et al., 2006 ; 2009) and degenerative diseases (Mollnau et al., 2006; Chen et al., 2007; Berrino, 2002; Meydani, 2002; Meydani, 2001). Encouraging the consumption of fruits and vegetables is now a major public health recommendation. Moreover, among the plant antioxidants, polyphenols appear to be the most effective in their protective effects. The polyphenols in *H. sabdariffa* were identified by a study conducted in our laboratory (Sarr et al., 2009) and many classes were found: phenolic, chlorogenic and caffeic acids, anthocyanins and flavonoids. This same study also found that polyphenols have a good vasorelaxant activity *in vitro*. What about their efficacy on tolerance to nitrates? The main results obtained during the present study have clearly demonstrated the interest of polyphenols on nitrate tolerance.

One important result obtained in our study is the potentiation of the relaxant responses to nitroglycerin with the *H. sabdariffa* extract, whatever the type of vessels, tolerant or not tolerant. Similarly, potentiating effects were also found with N-acetyl cysteine. If potentiating response of nitroglycerin by antioxidants such as N-acetyl cysteine have been reported in numerous studies (Munzel et al., 1989; Pizzulli et al., 1997; Watanabe et al., 1998a, 1998b), few data relate those of plant polyphenols, and no data was reported for the polyphenols of *H. sabdariffa*. To our knowledge, such results linking these potentiating effects on relaxation responses to nitroglycerin and polyphenols from *H. sabdariffa* is an original result.

Another important result is that these potentiating responses of the *H. sabdariffa* extract on the responses of nitroglycerin are responsible for the continuing state of tolerance. This result is explained by the fact that the HSE potentiate all types of vessels relaxed with nitroglycerin, which persist the state of tolerance. This result is very interesting because it can be a possibility to

significantly reduce the therapeutic doses of nitroglycerin and delay the development of tolerance. Similar results were also found with NAC but not with VIT C. These results are consistent with those reported by Pizzuli et al. (1997), showing that NAC does not prevent tolerance during nitroglycerin infusion in patients with heart failure. However, the concomitant infusion of NAC and NTG in 48 h continuous infusion attenuates the development of nitrate tolerance in patients with normal left ventricular function. Regarding vitamin C, Watanabe et al. (1998b) have also shown that oral administration of this anti-oxidant may prevent nitrate tolerance during continuous administration of NTG in patients with ischemic heart disease.

Finally, data obtained with apocynin, a polyphenols structural analogue, reported by Fukatsu et al. (2007) indicated that this compound, pharmacologically known to be able to prevent the increase in protein expression of NADPH oxidase, a source of superoxide anions, could be a means of protection against nitrate tolerance.

Conclusion

In the long-term treatment of angina pectoris, the difficulty observed is the gradual reduction of coronary arteries relaxant responses to nitrates, despite increasing doses. Our work performed to assess the potential of *H. sabdariffa* polyphenols suggests interesting therapeutic prospects. This could improve the coronary relaxation responses by potentiating the relaxing effect of nitroglycerin.

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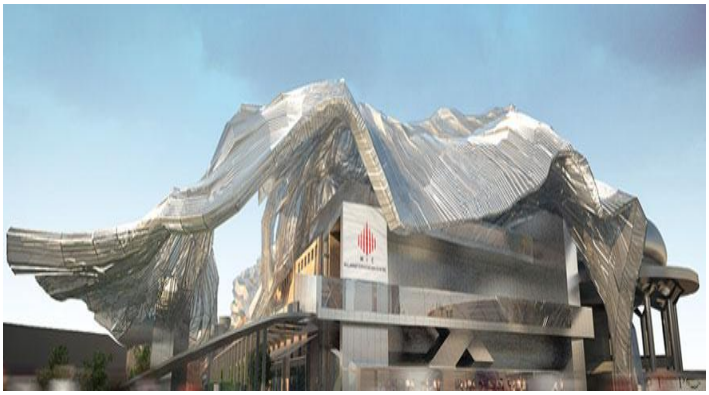

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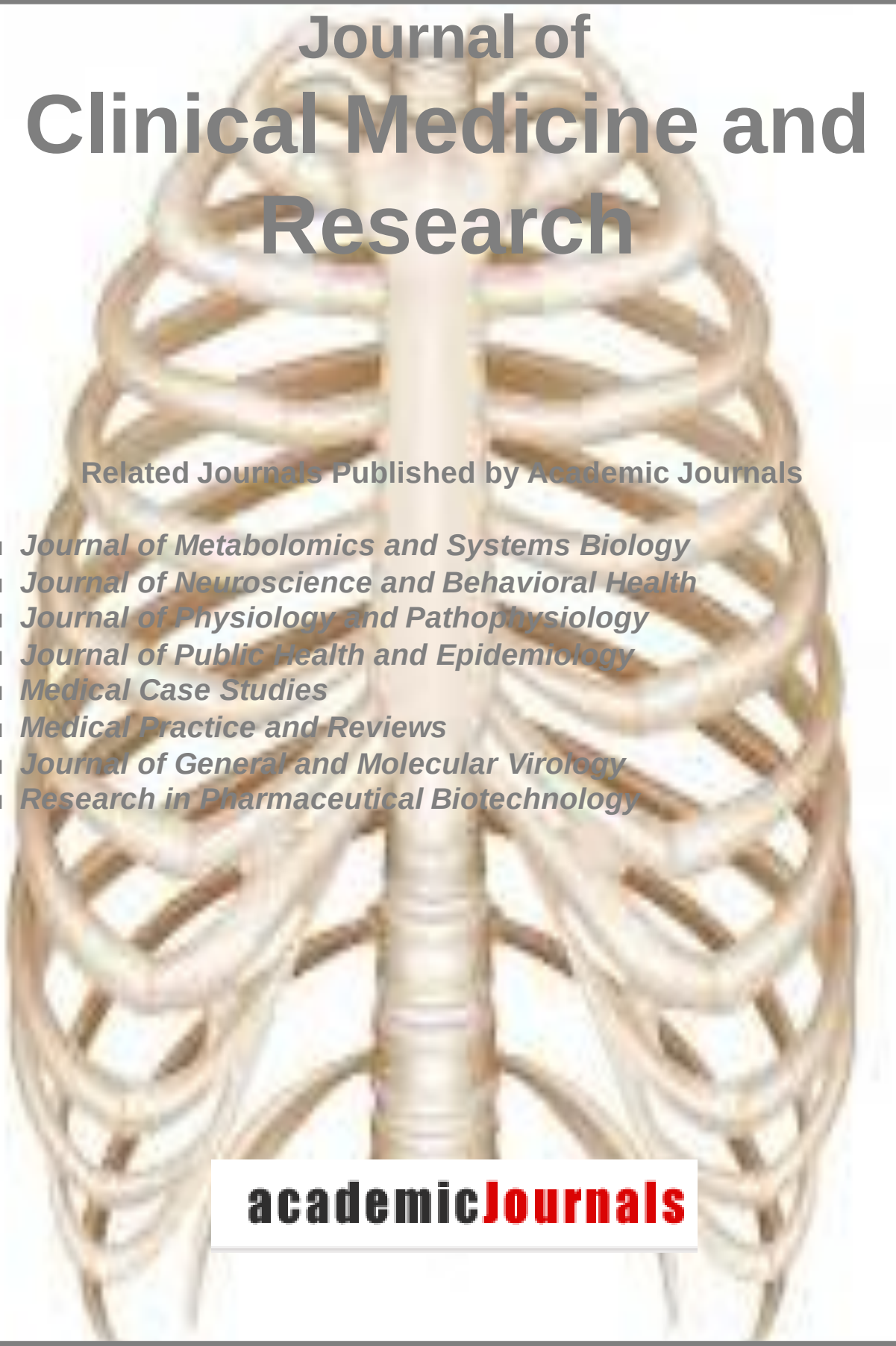

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