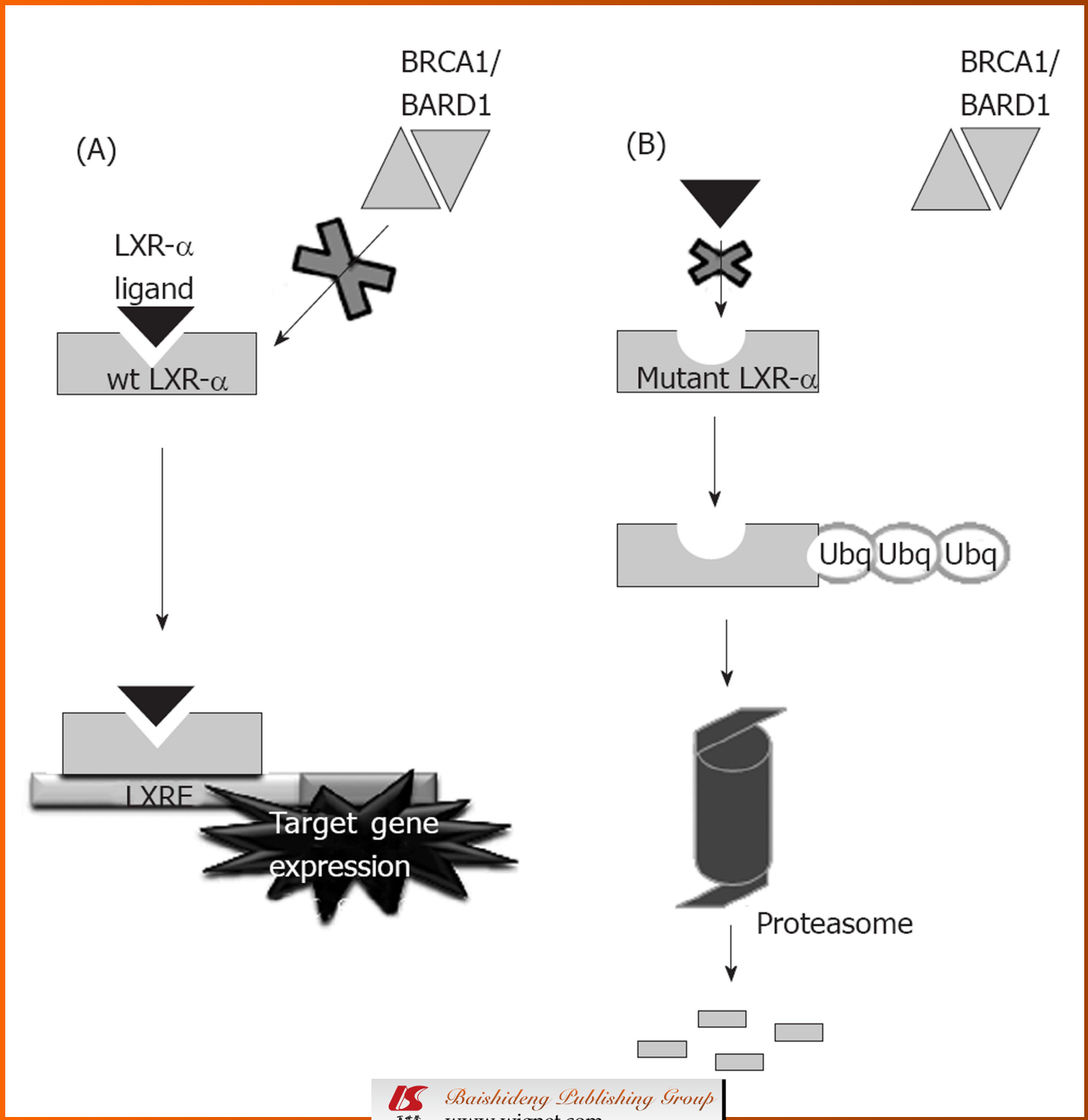
ISSN 1949-8462 (online)

# World Journal of *Cardiology*

*World J Cardiol* 2013 August 26; 5(8): 270-316




Baishideng Publishing Group
www.wjgnet.com

World Journal of Cardiology

A peer-reviewed, online, open-access journal of Cardiology

# Editorial Board

2009-2013

The *World Journal of Cardiology* Editorial Board consists of 362 members, representing a team of worldwide experts in cardiology. They are from 43 countries, including Argentina (4), Australia (9), Belgium (2), Brazil (5), Canada (24), Chile (1), China (18), Colombia (1), Czech (1), Denmark (4), France (3), Germany (32), Greece (14), Hungary (2), India (8), Iran (2), Ireland (1), Israel (2), Italy (44), Japan (24), Kosovo (1), Lebanon(1), Malaysia (1), Mexico (1), Morocco (1), Netherlands (9), Nigeria (1), Oman (1), Pakistan (1), Poland (3), Portugal (1), Russia (1), Singapore (1), Slovenia (2), South Africa (2), South Korea (6), Spain (10), Switzerland (1), Thailand (1), Turkey (8), United Kingdom (14), United States (93), and Uruguay (1).

**Denmark**

Morten Grunnet, *Ballerup*
Won Yong Kim, *Aarhus*
Ole Dyg Pedersen, *Copenhagen*
Jacob Tfelt-Hansen, *Copenhagen*

**France**

Philippe Commeau, *Ollioules*
Yves D Durandy, *Massy*
Thierry Lefèvre, *Massy*

**Germany**

Ferruh Artunc, *Tübingen*
Muhammet A Aydin, *Hamburg*
Alexander Bauer, *Heidelberg*
Peter Bernhardt, *Ulm*
Torsten Bossert, *Jena*
Marcus Dörr, *Greifswald*
Holger Eggebrecht, *Essen*
Tommaso Gori, *Mainz*
Dariusch Haghi, *Mannheim*
Stefan E Hardt, *Heidelberg*
Klaus Hertting, *Hamburg*
Thomas Jax, *Neuss*
Thorsten Kälsch, *Mannheim*
Klaus Kettering, *Frankfurt*
Grigorios Korosoglou, *Heidelberg*
Horst J Kuhn, *Planegg*
Lorenz H Lehmann, *Heidelberg*
Huige Li, *Mainz*
Veselin Mitrovic, *Bad Nauheim*
Ulrich Nellessen, *Stendal*
Guenter Pilz, *Hausham*
Peter W Radke, *Lübeck*
Obaida Rana, *Aachen*
Tienush Rassaf, *Düsseldorf*
Oliver Ritter, *Wuerzburg*
Erol Saygili, *Aachen*
Dirk Skowasch, *Bonn*
Tim Süselbeck, *Mannheim*
Dirk Taubert, *Cologne*
Theodor Tirilomis, *Goettingen*
Stephen Wildhirt, *Ulm*
Thomas Zeller, *Bad Krozingen*

**Greece**

Yiannis S Chatzizisis, *Thessaloniki*
Moses S Elisaf, *Ioannina*
Gerasimos Filippatos, *Athens*
Panagiotis Korantzopoulos, *Ioannina*
Nicholas G Kounis, *Patras*
Antigone Lazou, *Thessaloniki*
Konstantinos P Letsas, *Athens*
Athanassios N Manginas, *Athens*
Lampros Michalis, *Ioannian*
Serafim Nanas, *Athens*
Loukianos S Rallidis, *Athens*
Georgios I Tagarakis, *Thessaloniki*
Dimitrios Tziakas, *Alexandroupolis*
Theodoros Xanthos, *Athens*

**Hungary**

Gergely Feher, *Pecs*
Albert Varga, *Szeged*

**India**

MPS Chawla, *Roorkee*
S Dwivedi, *Delhi*
Rajeev Gupta, *Jaipur*
Deepak Kaul, *Chandigarh*
Prabhakaran Prabhakaran, *New Delhi*
KV Pugalendi, *Tamilnadu*
Rajesh Vijayvergiya, *Chandigarh*

**Iran**

VR Dabbagh Kakhki, *Mashhad*
Roya Kelishadi, *Isfahan*

**Ireland**

Jonathan D Dodd, *Dublin*

**Israel**

Jacob George, *Tel Aviv*
E Goldhammer, *Haifa*

**Italy**

Maria Grazia Andreassi, *Massa*
Giuseppe Barbaro, *Rome*
Riccardo Bigi, *Milan*
Tonino Bombardini, *Pisa*
Filippo Cademartiri, *Parma*
Alessandro Capucci, *Piacenza*
Sergio Coccheri, *Bologna*
Antonio Colombo, *Milan*
Alberto Cuocolo, *Napoli*
Roberto De Ponti, *Varese*
Gianluca Di Bella, *Messina*
Giovanni Fazio, *Palermo*
Vittorio Fineschi, *Foggia*
Antonio F Folino, *Padova*
Gabriele Fragasso, *Milano*
Carmine Gazzaruso, *Vigevano*
Massimo Imazio, *Torino*
Federico Lombardi, *Milan*
Roberto Marchioli, *Santa Maria Imbaro*
Giovan Giuseppe Mattera, *Pomezia*
Germano Melissano, *Milano*
Pietro A Modesti, *Florence*
Eraldo Occhetta, *Novara*
Pasquale Pagliaro, *Orbassano*
Emilio Maria G Pasanisi, *Pisa*
Vincenzo Pasceri, *Rome*
Salvatore Patanè, *Messina*
Nunzia Rosa Petix, *Florence*
Eugenio Picano, *Pisa*
Rita Rezzani, *Brescia*
Manfredi Rizzo, *Palermo*
Gian Paolo Rossi, *Padua*
Speranza Rubattu, *Rome*
Andrea Rubboli, *Bologna*
Rosa Sicari, *Pisa*
Giuseppe Tarantini, *Padua*
Luigi Tavazzi, *Cotignola*
Luca Testa, *Milan*
Maurizio Turiel, *Milan*
Cristina Vassalle, *Pisa*
Massimo Volpe, *Rome*

**Japan**

Yoshifusa Aizawa, *Niigata*
Junichiro Hashimoto, *Sendai*
Hajime Kataoka, *Oita*
Akinori Kimura, *Tokyo*
Sei Komatsu, *Amagasaki*
Ikuo Fukuda, *Hirosaki*
Satoshi Kurisu, *Hiroshima*
Yoshihiro Matsumoto, *Shizuoka*
Tetsuo Minamino, *Osaka*
Yoko Miyasaka, *Osaka*
Kenichi Nakajima, *Kanazawa*
Mashio Nakamura, *Tsu*
Kazuaki Nishio, *Tokyo*
Koichi Sakabe, *Kagawa*
Masataka Sata, *Tokushima*
Shinji Satoh, *Fukuoka*
Yoshihide Takahashi, *Kanagawa*
Masamichi Takano, *Chiba*
Kengo Tanabe, *Tokyo*
Hiroki Teragawa, *Hiroshima*
Hiroyasu Ueda, *Osaka*
Takanori Yasu, *Okinawa*
Hiroshi Yoshida, *Chiba*

**Kosovo**

Gani Bajraktari, *Prishtina*

**Lebanon**

Habib A Dakik, *Beirut*

**Malaysia**

Eric Tien Siang Lim, *Johor*

**Mexico**

Enrique Vallejo, *Mexico*

**Morocco**

Abdenasser Drighil, *Casablanca*

**Netherlands**

Folkert Wouter Asselbergs, *Groningen*
Jeroen J Bax, *Leiden*
JJ Brugts, *Rotterdam*
Peter W de Leeuw, *AZ Maastricht*
Corstiaan A Den Uil, *Rotterdam*
PA Doevendans, *Utrecht*
D Poldermans, *Rotterdam*
PW Serruys, *Rotterdam*

**Nigeria**

OS Ogah, *Ibadan*

**Pakistan**

Fahim H Jafary, *Karachi*

**Poland**

Pawel Buszman, *Katowice*
Maciej Kurpisz, *Poznan*
Sebastian Szmit, *Warsaw*

**Russia**

Nadezda Bylova, *Moscow*

**Singapore**

Jinsong Bian, *Singapore*

**Slovenia**

Mitja Lainscak, *Golnik*
Matej Podbregar, *Ljublajna*

**South Africa**

Benjamin Longo-Mbenza, *Pretoria*
JP Smedema, *Capetown*

**South Korea**

Jang-Ho Bae, *Daejeon*
Young-Guk Ko, *Seoul*
Sang-Hak Lee, *Seoul*
Pil-Ki Min, *Seoul*
Seung-Jung Park, *Seoul*

**Spain**

Miguel A Arias, *Toledo*
Antoni Bayés-Genís, *Barcelona*
Alberto Dominguez-Rodriguez, *Tenerife*
Lorenzo Facila, *Castellon*
José Luis Pérez-Castrillon, *Valladolid*
Jesus Peteiro, *Coruña*
Pedro L Sánchez, *Madrid*
José L Zamorano, *Madrid*

**Switzerland**

Paul Erne, *Luzern*

**Thailand**

Nipon Chattipakorn, *Chiang Mai*

**Turkey**

Turgay Çelik, *Etlik-Ankara*
Yengi U Celikyurt, *Kocaeli*
Hamza Duygu, *Yesilyurt*
Cemil Gürgün, *İzmir*
T Fikret Ilgenli, *Kocaeli*
Ergün Barış Kaya, *Ankara*
Mehmet Ozaydin, *Isparta*
Mustafa Yildiz, *Istanbul*

**United Kingdom**

AD Blann, *Birmingham*
Geoffrey Burnstock, *London*
John GF Cleland, *Kingston upon Hull*
Armen Yuri Gasparyan, *Dudley*
Derek J Hausenloy, *London*
Farhad Kamali, *Newcastle upon Tyne*
Juan Carlos Kaski, *London*
Rajesh G Katare, *Bristol*
Sohail Q Khan, *Manchester*
Khalid Rahman, *Liverpool*
Alexander M Seifalian, *London*
Mark Slevin, *Manchester*
Anastasis Stephanou, *London*

**United States**

Kamran Akram, *Omaha*
Arshad Ali, *Ashland*
Mouaz Al-Mallah, *Detroit*
Naser M Ammash, *Rochester*
Vignendra Ariyarajah, *Philadelphia*
Wilbert S Aronow, *Valhalla*
S Serge Barold, *Tampa*
Gregory W Barsness, *Rochester*
Daniel S Berman, *Los Angeles*
John F Beshai, *Chicago*
William E Boden, *Buffalo*
Somjot S Brar, *Los Angeles*
David W Brown, *Decatur*
Lu Cai, *Louisville*
Christopher Paul Cannon, *Boston*
Ricardo Castillo, *Brooklyn*
Jun R Chiong, *Loma Linda*
Steven G Chrysant, *Oklahoma*
Timm Dickfeld, *Baltimore*
Dayue Darrel Duan, *Reno*
Rosemary B Duda, *Boston*
Michael E Farkouh, *New York*
Arthur Michael Feldman, *Philadelphia*
Ronald Freudenberger, *Allentown*
Jalal K Ghali, *Detroit*
Lev G Goldfarb, *Bethesda*
Samuel Z Goldhaber, *Boston*
Hitinder S Gurm, *Ann Arbor*
Julia H Indik, *Tucson*
Antony Leslie Innasimuthu, *Pittsburgh*
Ami E Iskandrian, *Birmingham*
Rovshan M Ismailov, *Pittsburgh*
Diwakar Jain, *Philadelphia*
Shahrokh Javaheri, *Mason*
Jacob Joseph, *West Roxbury*
Bobby V Khan, *Atlanta*
Christopher M Kramer, *Charlottesville*
Rakesh C Kukreja, *Richmond*
Roberto M Lang, *Chicago*
Marzia Leacche, *Nashville*
Jingping Lin, *Bethesda*
Yi-Hwa Liu, *New Haven*
Angel López-Candales, *Pittsburgh*
Frank Marcus, *Tucson*
Malek G Massad, *Chicago*
Jawahar L Mehta, *Little Rock*
Robert M Mentzer Jr, *Detroit*
J Gary Meszaros, *Rootstown*
Michael Miller, *Baltimore*
Emile R Mohler III, *Philadelphia*
Patrick M Moriarty, *Kansas City*
Jeffrey W Moses, *New York*
Mohammad-Reza Movahed, *Tucson*
Gerald V Naccarelli, *Hershey*
Andrea Natale, *Austin*
Tien MH Ng, *Los Angeles*
Steven Nissen, *Cleveland*
Gian M Novaro, *Weston*
Brian Olshansky, *Iowa*
Robert Lee Page II, *Aurora*
Weihong Pan, *Baton Rouge*
Linda Pauliks, *Hershey*
Philip Jack Podrid, *Boston*
Vikas K Rathi, *Midlothian*
Jun Ren, *Laramie*
Harmony R Reynolds, *New York*
Clive Rosendorff, *Bronx*
Samir Saba, *Pittsburgh*
Rajesh Sachdeva, *Little Rock*
Sandeep A Saha, *Spokane*
Tiziano M Scarabelli, *Detroit*
Robert H Schneider, *Maharishi Vedic*
Frank W Sellke, *Providence*
Samin K Sharma, *New York*
Jamshid Shirani, *Danville*
Boris Z Simkhovich, *Los Angeles*
Krishna Singh, *Johnson City*
Laurence S Sperling, *Atlanta*
Jonathan S Steinberg, *New York*
Ernst R von Schwarz, *Los Angeles*
Richard Gary Trohman, *Chicago*
Tong Tang, *San Diego*
Qing Kenneth Wang, *Cleveland*
Yi Wang, *Wilmington*
Adam Whaley-Connell, *Columbia*
Bruce L Wilkoff, *Cleveland*
Qinglin Yang, *Birmingham*
Xing Sheng Yang, *Atlanta*
Yucheng Yao, *Los Angeles*
Midori A Yenari, *San Francisco*
Cuihua Zhang, *Columbia*

**Uruguay**

Juan C Grignola, *Montevideo*

World Journal of Cardiology

Contents

Monthly Volume 5 Number 8 August 26, 2013

*World Journal of Cardiology*
**Volume 5 Number 8 August 26, 2013**

## Contents





NAME OF JOURNAL
*World Journal of Cardiology*

ISSN
ISSN 1949-8462 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly



EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
*World Journal of Cardiology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjc@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
August 26, 2013



INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1949-8462/g_info_20100316161927.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.270

*World J Cardiol* 2013 August 26; 5(8): 270-279
ISSN 1949-8462 (online)


REVIEW

# Early detection of cardiac involvement in thalassemia: From bench to bedside perspective

Nut Koonrungsesomboon, Siriporn C Chattipakorn, Suthat Fucharoen, Nipon Chattipakorn

Nut Koonrungsesomboon, Siriporn C Chattipakorn, Nipon Chattipakorn, Cardiac Electrophysiology Research and Training Center, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
Nut Koonrungsesomboon, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
Siriporn C Chattipakorn, Department of Oral Biology and Diagnostic Science, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand
Suthat Fucharoen, Thalassemia Research Center, Institute of Molecular Medicine, Mahidol University, Bangkok 3310, Thailand
Author contributions: Koonrungsesomboon N, Chattipakorn SC, Fucharoen S and Chattipakorn N solely contributed to this paper.
Supported by Thailand Research Fund grants RTA5580006 and BRG5480003
Correspondence to: Nipon Chattipakorn, MD, PhD, Cardiac Electrophysiology Research and Training Center, Department of Physiology, Faculty of Medicine, Chiang Mai University, Huay Kaew Road, Tambon Suthep, Muang District, Chiang Mai 50200, Thailand. nchattip@gmail.com
Telephone: +66-53-945329 Fax: +66-53-945329
Received: July 9, 2013 Revised: July 31, 2013
Accepted: August 5, 2013
Published online: August 26, 2013

## Abstract

Myocardial siderosis is known as the major cause of death in thalassemia major (TM) patients since it can lead to iron overload cardiomyopathy. Although this condition can be prevented if timely effective intensive chelation is given to patients, the mortality rate of iron overload cardiomyopathy still remains high due to late detection of this condition. Various direct and indirect methods of iron assessment, including serum ferritin level, echocardiogram, non-transferrin-bound iron, cardiac magnetic resonance T2*, heart rate variability, and liver biopsy and myocardial biopsy, have been proposed for early detection of cardiac iron overload in TM patients. However, controversial evidence and limitations of their use in clinical practice exist. In this review article, all of these iron assessment methods that have been proposed or used to directly or indirectly determine the cardiac iron status in TM reported from both basic and clinical studies are comprehensively summarized and presented. Since there has been growing evidence in the past decades that cardiac magnetic resonance imaging as well as cardiac autonomic status known as the heart rate variability can provide early detection of cardiac involvement in TM patients, these two methods are also presented and discussed. The existing controversy regarding the assessment of cardiac involvement in thalassemia is also discussed.



**Key words:** Thalassemia; Iron overload; Cardiomyopathy; Serum ferritin; Heart rate variability; Magnetic resonance; Non-transferrin-bound iron

**Core tip:** The mortality of thalassemia major (TM) patients due to iron overload cardiomyopathy is still high even though it can be prevented with effective chelation. The role of reliable methods to determine cardiac iron status is very important in order to give a timely effective treatment. This review article provides a comprehensive summary and discussion of various iron assessment methods as well as their existing controversy for use from both basic and clinical reports that have been proposed or used to directly or indirectly determine the cardiac iron status in TM.

Koonrungsesomboon N, Chattipakorn SC, Fucharoen S, Chattipakorn N. Early detection of cardiac involvement in thalassemia: From bench to bedside perspective. *World J Cardiol* 2013; 5(8): 270-279 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/270.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.270

## INTRODUCTION

Thalassemia major (TM) is an inherited anemia caused by impaired synthesis of the beta goblin chain. The prevalence of thalassemia is high in the Mediterranean countries, the Middle East, Central Asia, India, Southern China and Thailand[1]. Approximately 60000 TM infants are reportedly born each year[2]. Due to severe hemolytic anemia, TM patients need to habitually receive blood transfusions beginning in infancy. Regular blood transfusions, increased intestinal iron absorption as well as the lack of active excretion of iron inevitably lead to an excess accumulation of iron in the body of TM patients including not only in the reticuloendothelial cells, but also in the parenchymal tissues as well[3]. Excess free iron participating in the Fenton-type reaction has been shown to contribute to the pathogenesis of hemochromatosis[4]. Among many complications due to iron overload, myocardial siderosis is the major cause of mortality in these TM patients[5].

At present, although bone marrow transplantation has been shown to effectively cure some selected patients, the cornerstone of treatment in TM is still with blood transfusion and iron chelation therapy. The effectiveness of iron chelation has markedly improved since the introduction of oral chelators, such as deferiprone[6] and deferasirox[7], resulting in prolonged life expectancy and increased quality of life in TM patients. Despite the effectiveness of iron chelators, iron overload cardiomyopathy can be reversible only if early intensive chelation has been initiated[8,9]. Once TM patients develop clinical symptom such as heart failure or arrhythmia, the prognosis usually becomes poor and death thereafter in spite of intensive chelation[10]. These findings indicate the importance of early detection of cardiac iron accumulation prior to the development of cardiac dysfunction, and that the intensive chelation can be given promptly to those patients who are at risk. Currently, various methods for the detection of cardiac involvement in iron overload condition have been reported both in animal models as well as in clinical studies. Nevertheless, there are still limitations of their use in TM patients due to controversial reports on their reliability or limited access to the machine used for the detection as well as their high cost. In this review article, various methods that have been proposed or used to directly or indirectly determine the cardiac iron status in TM reported from basic and clinical studies are comprehensively summarized and presented. The existing controversy regarding the assessment of cardiac involvement in thalassemia is also discussed.

## ASSESSMENT OF CARDIAC INVOLVEMENT IN THALASSEMIA

Since clinical evaluation is unreliable to detect an early stage of iron overload cardiomyopathy in TM patients, several approaches have been used to determine cardiac iron status instead. These include the indirect cardiac iron assessment such as serum ferritin, echocardiogram, and electrocardiogram (ECG) as well as the direct but invasive assessment such as myocardial biopsy and liver biopsy. Since there has been growing evidence in the past decades that cardiac magnetic resonance imaging (MRI) as well as cardiac autonomic status known as the heart rate variability (HRV) can provide early detection of cardiac involvement in TM patients, these two methods will also be presented and discussed.

### *Serum ferritin*

Serum ferritin has been used for decades as a predictor of iron overload status in clinical practice due to its strong correlation with hepatic iron[11], representing an indirect index for estimating the total body iron stores. It is inexpensive and accessible worldwide. Serum ferritin has been shown to have a positive relationship with the amount of blood transfusion in beta-thalassemia patients[12]. Furthermore, it has been shown that a serum ferritin level greater than 1800 μg/L was associated with the increased concentration of cardiac iron, and that serum ferritin greater than 2500 μg/L was associated with the increased prevalence of cardiac events[13].

The downturn of using serum ferritin as an assessment of iron overload is due to the fact that the increased level of serum ferritin is not specific to iron overload condition since its level can also be increased in other conditions such as inflammation, collagen diseases, hepatic diseases, and malignancy[14]. Evidence indicated that an increased serum ferritin levels might be a defense mechanism of the body against oxidative stress[15]. Moreover, a low serum ferritin level does not necessarily designate low risk of iron-induced cardiomyopathy[16]. Several studies in the last decade demonstrated that serum ferritin is not suitable for its use as a predictive indicator of myocardial iron deposition due to its lack of relationship with cardiac iron[17,18]. A recent study reported that many unexplained cardiac deaths in TM patients were found even though they had low serum ferritin levels[19], emphasizing the unreliable use of serum ferritin as a predictor for iron overload cardiomyopathy in TM patients.

### *Echocardiogram*

Echocardiogram is a valuable tool for cardiac function monitoring in clinical practice. However, several studies demonstrated that it is not sensitive enough for early detection of the preclinical stage of cardiac involvement in TM patients due to the typical late onset of symptoms and signs[20]. Once cardiac dysfunction is detected by an echocardiogram, the survival rate of these patients is reduced[21,22], suggesting a late stage detection of the disease by this assessment. In addition, it has been shown that the absence of a reduced left ventricular ejection fraction (LVEF) does not exclude a significant risk of sudden potential cardiac decompensation from iron overload[23]. Since left ventricular function is often slightly higher than normal in thalassemia patients in the absence of

myocardial iron overload[24], the normal values of cardiac function by echocardiogram may not be able to rule out cardiac impairment by iron deposition in these patients. Therefore, routine monitoring of cardiac function by echocardiogram is not reliable in early detecting thalassemia patients with high risk of cardiac involvement in order to provide timely intensive treatment.

### Electrocardiogram

Since most of TM patients with early cardiac involvement are asymptomatic, ECG has no value for screening of cardiac involvement in this group of patients[25]. Similar to echocardiogram, once the development of cardiac arrhythmias, such as premature atrial or ventricular contractions, first-degree atrioventricular block, atrial flutter, atrial fibrillation, ventricular tachycardia, and second-degree or complete heart block[26-28], is detected by ECG, it usually implies an advanced stage of disease[29,30]. Furthermore, a normal ECG does not exclude a risk of significant arrhythmia development in iron overload patients[25]. In a retrospective analysis, which included 27 transfusion-dependent thalassemia patients who underwent annual 24-h electrocardiographic monitoring, two patients developed significant clinical symptoms secondary to cardiac arrhythmias within one year of follow-up[31]. This result indicated that a 24-h electrocardiogram might be useful for arrhythmia detection, but is not totally predictive for life-threatening cardiac events. Therefore, both ECG and conventional 24-h ECG monitoring are not appropriate markers for early detection of cardiac involvement in thalassemia patients.

### Liver and myocardial biopsy

Liver biopsy is a direct determination of liver iron concentration closely reflecting total body iron storage[32]. However, a previous study demonstrated that hepatic iron concentration correlates poorly with cardiac iron status and cardiac function[33]. These findings indicated that determination of iron level *via* liver biopsy does not reflect cardiac iron deposition. Moreover, this technique is an invasive procedure that is not suitable for regular monitoring of iron status in thalassemia patients.

A previous study has also shown that iron level determined by an invasive myocardial biopsy was not correlated with cardiac iron status and cardiac function[34]. This could be due to the fact that myocardial iron deposition was inhomogeneous in the heart[35]. As a result, myocardial biopsy is not recommended to be used as an indicator for cardiac iron overload assessment.

### Superconducting quantum interference device

Superconducting quantum interference device (SQUID) biomagnetic liver susceptometry (BLS) has become a standard method in monitoring iron in the liver[36,37]. However, it has many limitations including its availability, cost, technical demands, and suboptimal reproducibility[38]. Together with the lack of heart data, SQUID has not been recommended for its use in the evaluation of cardiac iron status in patients with thalassemia.

### Non-transferrin-bound iron

Non-transferrin-bound iron (NTBI), a free-form iron, can be detected in plasma when the iron binding capacity of transferrin is saturated[39]. This form of iron is able to generate free radical *via* the Fenton-type reactions, leading to peroxidative damage to membrane lipid and protein[40]. The rate of NTBI uptake into cells is approximately 300-fold greater than that of transferrin-bound iron[41] due to its independence on the presence of transferrin receptor[42] and none of feedback-regulated process[43]. Moreover, there is a positive correlation between the rate of NTBI uptake and cellular iron content[44]. Furthermore, a recent study demonstrated a direct correlation between NTBI and vital organ damage in thalassemia patients[45]. In a normal individual, there is no detectable NTBI[46]; on the other hand, hemochromatosis patients exhibit higher NTBI levels than controls[47]. The growing evidence on NTBI suggests that it could be a good index of iron overload in TM patients.

Despite these facts, currently there is neither a cut-point threshold to imply cardiac iron overload status nor even a universally accepted method for NTBI measurement at the present time[48]. Importantly, a poor correlation was found between the methods in a recent inter-laboratory survey[49]. As a consequence, these limitations minimize its use in clinical practice.

### Cardiac magnetic resonance T2*

Cardiac magnetic resonance T2* (CMR T2*) has become a widely used tool for its accurate and non-invasive technique to measure iron deposition in heart[50]. Currently, this technique has been proven to be the most sensitive index and reproducible to assess cardiac iron available today[50,51]. Anderson *et al*[16] first reported a significant relationship between myocardial T2* below 20 ms and cardiac function parameters, such as LVEF ($r = 0.61$, $P < 0.0001$), left ventricular (LV) end-systolic volume index ($r = 0.50$, $P < 0.0001$), and LV mass index ($r = 0.40$, $P < 0.001$). A later study confirmed the correlation of myocardial T2* with not only systolic function but also diastolic function as well[52]. Moreover, an increase of myocardial T2* was also in accordance with improved cardiac function[17]. Previous studies in a fresh postmortem iron overloaded heart[53] and a gerbil model of iron overload[54] clearly demonstrated a negative correlation between CMR T2* values and myocardial iron deposition. It also confirmed the earlier studies that iron loading was deposited mostly in the epicardium and myocardium[35,55]. Until now, no clinical scenario other than cardiac iron overload is found to cause myocardial T2* below 20 ms[50]. Thus, these data implied that CMR T2* is more specific to cardiac iron status than other previously mentioned methods.

The prospective study by Kirk *et al*[56] indicated the significant strong association between cardiac T2* values and risk of heart failure development in TM patients. It

**Table 1 Summary of the controversial correlation between cardiac magnetic resonance T2* and serum ferritin in thalassemia major**

| Population/size | Type of study | Findings | Correlation | Ref. |
|---|---|---|---|---|
| TM/652 patients | Prospective | Significant correlation between cardiac T2* and ferritin ($r^2$ = 0.003, $P$ = 0.04) | / | Kirk *et al*[56] |
| TM/776 patients | Retrospective | Significant relationship between cardiac R2* and ferritin ($r$ = -0.359, $P$ < 0.0001) | / | Marsella *et al*[59] |
| TM/167 patients | Prospective | Myocardial T2* was correlated with serum ferritin ($r$ = -0.34, $P$ < 0.001) | / | Tanner *et al*[60] |
| TM/19 patients, SCD/17 patients | Cross sectional | Cardiac 1/T2* was correlated with ferritin level ($r^2$ = 0.33, $P$ = 0.01) | / | Wood *et al*[61] |
| TM/106 patients | Prospective | No significant correlation between heart T2* and serum ferritin | × | Anderson *et al*[16] |
| TM/60 patients | Prospective | Serum ferritin did not correlate with cardiac iron values | × | Merchant *et al*[57] |
| TM/20 patients | Prospective | No correlation between serum ferritin and cardiac T2* | × | Kolnagou *et al*[58] |
| TM/47 patients | Retrospective | Cardiac T2* was not associated with the serum ferritin | × | Bayraktaroğlu *et al*[22] |

TM: Thalassemia major; SCD: Sickle cell disease.

**Table 2 Summary of the correlation between cardiac magnetic resonance T2* and cardiac function in thalassemia major**

| Population/size | Type of study | Findings | Correlation | Ref. |
|---|---|---|---|---|
| TM/776 patients | Retrospective | Significant correlation between LVEF and cardiac R2* ($r$ = -0.327, $P$ < 0.0001) | / | Marsella *et al*[59] |
| TM/106 patients | Prospective | Significant correlation of myocardial T2* below 20 ms with LVEF ($r$ = 0.61, $P$ < 0.0001), LVESVi ($r$ = 0.50, $P$ < 0.0001), and LV mass index ($r$ = 0.40, $P$ < 0.001) | / | Anderson *et al*[16] |
| TM/167 patients | Prospective | Significant relationship between myocardial iron and LVEF ($r$ = 0.57, $P$ < 0.001) | / | Tanner *et al*[60] |
| TM/67 patients | Cross sectional | Myocardial T2* related to LV diastolic function (EPFR, $r$ = –0.20, $P$ = 0.19; APFR, $r$ = 0.49, $P$ < 0.001; EPFR/APFR ratio, $r$ = –0.62, $P$ < 0.001) | / | Westwood *et al*[52] |
| TM/33 patients | Cross sectional | Good correlation of DT, Tei index and E/Em index with cardiac T2* values ($P$ < 0.05, $r$ = 0.70-0.81) and weak correlation of E/A with T2* ($P$ < 0.05, $r$ = -0.44) | / | Barzin *et al*[84] |
| TM/47 patients | Retrospective | Significant correlations of the myocardial T2* with LVESVi and LVEDVi ($r$ = -0.32, $P$ = 0.027; $r$ = -0.29, $P$ = 0.046, respectively) | / | Bayraktaroğlu *et al*[22] |
| TM/19 patients, SCD/17 patients | Cross sectional | Significant relationship between LVEF and myocardial T2* | / | Wood *et al*[61] |

TM: Thalassemia major; SCD: Sickle cell disease; LVEF: Left ventricular ejection fraction; LVESVi: Left ventricular end systolic volume index; LVEDVi: Left ventricular end diastolic volume index; EPFR: Early peak filling rate; APFR: Atrial peak filling rate; DT: Deceleration time; E/Em: Early diastolic peak inflow velocity and early diastolic myocardial velocity ratio; E/A: Early and late transmittal peak flow velocity ratio.

demonstrated that 98% of patients who developed heart failure had the cardiac T2* less than 10 ms, with a relative risk (RR) of 160 (95%CI: 39-653). In the same study, the RR for cardiac T2* less than 6 ms was 270 (95%CI: 64-1129). Moreover, T2* threshold of 10 ms for predicted heart failure had a sensitivity of 97.5% (95%CI: 91.3-99.7) and a specificity of 85.3% (95%CI: 83.3-87.2). This study also demonstrated the significant relationship between cardiac T2* values and a risk of cardiac arrhythmia development in TM patients, but weaker than a risk of heart failure. A cardiac T2* less than 20 ms was figured in 83% of patients who develop arrhythmia, with a RR of 4.60 (95%CI: 2.66-7.95). The RR for a cardiac T2* less than 6 ms was 8.79 (95%CI: 4.03-19.2). The T2* threshold of 20 ms for predicted cardiac arrhythmia had a sensitivity of 82.7% (95%CI: 73.7-89.6) and a specificity of 53.5% (95%CI: 50.8-56.2). In addition, this prospective study clearly demonstrated the link between myocardial T2* and cardiac events. The one year risk of heart failure development was shown to be 14%, 30%, and 50% for T2* between 8-10, 6-8 and less than 6 ms, respectively. Therefore, myocardial T2* less than 10 ms strongly indicated clinically significant cardiac iron overload and an increase in risk of developing heart failure in TM patients.

When compared with conventional iron monitoring parameters, the correlation between CMR T2* and serum ferritin in TM patients has not been concluded (Table 1). Several studies indicated that serum ferritin was not correlated with cardiac T2*[16,22,57,58]. However, other studies with larger population size showed a weak relationship between serum ferritin and heart T2*[56,59-61]. Because serum ferritin is raised even in many common conditions such as inflammation or hepatic disease[14], the controversial correlation could be from subjects with a different underlying status included in each study. As a result, a guideline for intensive chelation therapy based on serum ferritin may be inappropriate for cardiological management in TM patients.

A prospective study of Tanner *et al*[62], which recruited 167 TM patients, showed the significant association between heart T2* values and LVEF. Patients with mild, moderate and severe cardiac iron overload (T2* 12-20, 8-12 and less than 8 ms, respectively) had impaired LVEF in

**Table 3 Comparison of various methods to evaluate cardiac iron overload in thalassemia patients**

| Method | Advantages | Disadvantages |
|---|---|---|
| Serum ferritin | Easy and available<br>Inexpensive | Poor predictor of iron overload[85,86]<br>Nonspecific for cardiac iron<br>Altered by many conditions[14] |
| Echocardiogram | Easy and available<br>Inexpensive | Late indicator of cardiac involvement[21,23] |
| Liver biopsy | Total body iron estimation[32] | Invasive<br>No correlation with myocardial iron deposition[33] |
| Myocardial biopsy | | Invasive<br>No correlation with cardiac iron status and function[34] |
| ECG | Easy and available<br>Inexpensive | Ineffective screening parameter for cardiac iron overload[25,31] |
| SQUID | Standardized noninvasive index for liver iron[36] | Lack of availability, technical demands, and reproducibility<br>Costly |
| NTBI | Direct parameter of freeform iron resulting in peroxidative damage[87] | Application for the study of heart iron pending<br>Limited availability<br>No generally accepted method[48], and poor correlation between methods[49] |
| CMR T2* | Method of choice for the assessment of tissue iron deposition in last decade[51]<br>Noninvasive measurement of cardiac iron deposition[50]<br>Available<br>High sensitivity and reproducible[50]<br>Correlation with clinical outcome[16,17,56,62,63] | Costly |

ECG: Electrocardiogram; SQUID: Superconducting quantum interference device; NTBI: Non-transferrin-bound iron; CMR T2*: Cardiac magnetic resonance T2*.

5%, 20% and 62%, respectively ($P < 0.001$). Table 2 summarized studies that showed the significant correlation between CMR T2* and cardiac function in TM patients. These studies suggest that myocardial T2* could be a useful application to determine cardiac iron overload tending to deteriorate cardiac function. As a result, CMR T2* may be suitable for use as an assessment of cardiac iron deposit in thalassemia patients for early detection of the cardiac iron status before the detection of clinical signs and symptoms of iron overload cardiomyopathy.

Since several studies showed a remarkably strong correlation of heart T2* value with clinical cardiac complications, including heart failure and arrhythmia, CMR T2* had been applied to monitor cardiac iron deposition in TM patients in UK[63,64]. Interestingly, the mortality rate was significantly reduced. Nowadays, CMR T2* is recognized as the method of choice for evaluation of cardiac iron deposition in TM patients[51]. However, the limitation of this technique is its rather expensive cost and only limited medical centers around the world are equipped with this technique.

The pros and cons of different approaches that monitor cardiac iron overload condition in thalassemia patients are summarized in Table 3.

## HRV IN THALASSEMIA MAJOR

HRV is used to indicate the variation over time of the period between successive heartbeats and determine cardiac autonomic function and overall cardiac health[65]. HRV analysis has been used to determine the cardiac autonomic function in patients with post-myocardial infarction[66,67]. Reduced HRV parameters were associated with a significant increased mortality in these patients[68,69]. A prospective study indicated that HRV analysis on 1-year post-myocardial infarction follow-up patients also had prognostic significance[70]. Furthermore, HRV parameters have been shown to a strong predictor of mortality in patients with heart failure[71,72], cardiac transplantation[73], and diabetic neuropathy[74].

Due to its non-invasiveness and easy derivation, HRV has been investigated as one of the promising parameters to initially detect cardiac involvement and has been widely studied in thalassemia in the last decades. A number of studies on HRV in TM patients have been reported since Franzoni *et al*[75] first proposed that HRV was depressed in TM patients. A summary of previous studies that exhibited the significantly reduced HRV parameters in TM patients and thalassemic mice is described in Table 4. All of previous studies reported that HRV parameters were reduced both in TM patients and thalassemic mice, indicating that thalassemic condition exerted some degrees of cardiac autonomic dysfunction. A recent study which investigated autonomic function by six quantitative autonomic function tests demonstrated that the prevalence of subclinical autonomic function impairment was higher in thalassemia patients compared to controls[76]. This result confirmed that thalassemia patients have autonomic dysfunction in some degree. In prospective studies by Kardelen *et al*[77] and De Chiara *et al*[78], no evidence of abnormal echocardiographic finding was shown in TM patients with reduced HRV. Therefore, a significantly reduced HRV could be an early indicator of preclinical stage of heart disease in TM group. Nevertheless, the evidence of HRV in TM patients has not been extensively

**Table 4 Summary of heart rate variability findings from both clinical and basic studies in thalassemia**

| Population/size | Type of study | Findings | References |
|---|---|---|---|
| 34 TM patients and 20 healthy subjects | Prospective | Significantly depressed both time and frequency domain HRV parameters in TM patients | Rutjanaprom *et al*[20] |
| 32 TM patients and 46 control subjects | Prospective | Significantly reduced all HRV parameters in TM patients | Kardelen *et al*[77] |
| 19 TM patients and 19 healthy volunteers | Cross sectional | Significantly lower both time and frequency domain HRV parameters in the TM group | Franzoni *et al*[75] |
| 100 TM patients and 60 healthy controls | Cross sectional | Lower SDNN in TM with ectopia while markedly increased LF/HF ratio in this group. | Oztarhan *et al*[88] |
| 48 Thalassemia patients and 45 healthy subjects | Cross sectional | Significantly reduced time domain parameters in the thalssemia group | Gurses *et al*[89] |
| 9 TM patients and 9 healthy subjects | Cross sectional | Significantly lower LF/HF ratio during tilt in TM patients than in control subjects | Veglio *et al*[90] |
| 21 TM patients and 15 healthy subjects | Cross sectional | Significantly lower in all HRV parameters in TM group than in control group | Ma *et al*[91] |
| 13 wildtype, 13 HbE/β thalassemia and 13 *mu*β+/− mice | Cross sectional | Depressed all HRV parameters in the heterozygous βglobin knockout mice (*mu*β+/−) | Incharoen *et al*[92] |
| 810 wildtype and 810 heterozygous betaknockout mice | Prospective | Higher LF/HF ratio in thalassemic mice than those in the wild type | Kumfu *et al*[82] |
| 12 wildtype and 12 heterozygous betaknockout mice | Prospective | Depressed HRV in betathalassemic mice compared to wild type | Thephinlap *et al*[93] |

TM: Thalassemia major; HRV: Heart rate variability; SDNN: Standard deviation of all NN intervals; LF: Low frequency power; HF: High frequency power.

**Table 5 Summary of the correlation between HRV and serum ferritin in thalassemia major**

| Population/size | Type of study | Findings | Correlation | References |
|---|---|---|---|---|
| 34 TM patients and 20 healthy subjects | Prospective | No correlations between HRV parameters and serum ferritin | × | Rutjanaprom *et al*[20] |
| 19 TM patients and 19 healthy volunteers | Cross sectional | No correlation between HRV parameters and serum ferritin | × | Franzoni *et al*[75] |
| 21 TM patients and 15 healthy subjects | Cross sectional | No relationship of HRV parameters with serum ferritin | × | Ma *et al*[91] |

TM: Thalassemia major; HRV: Heart rate variability.

**Table 6 Summary of the relationship between heart rate variability and cardiac function in thalassemia major**

| Population/size | Type of study | Findings | Correlation | References |
|---|---|---|---|---|
| 34 TM patients and 20 healthy subjects | Prospective | None of the echocardiographic parameters was correlated with HRV | × | Rutjanaprom *et al*[20] |
| 32 TM patients and 46 control subjects | Prospective | Reduced HRV were described in TM despite no echocardiographic abnormality | × | Kardelen *et al*[77] |
| 19 TM patients and 19 healthy volunteers | Cross sectional | No correlation between HRV parameters and echocardiographic parameters | × | Franzoni *et al*[75] |
| 20 TM patients | Prospective | Abnormal HRV in TM with no evidence of ventricular dysfunction | × | De Chiara *et al*[78] |

TM: Thalassemia major; HRV: Heart rate variability.

investigated when compared to that in post-myocardial infarction patients. Until now, none of studies has focused on the association between HRV and mortality in TM patients.

After the first report of HRV in TM patients by Franzoni *et al*[75], several studies have examined HRV in TM patients in order to seek the correlation between HRV and currently used iron overload parameters. No correlation between HRV parameters and serum ferritin in TM patients has been demonstrated (Table 5). Moreover, no correlation between HRV parameters and cardiac function in TM patients has been shown (Table 6). It is possible that HRV is not correlated with iron overload condition because several anemic diseases other than thalassemia, including sickle cell anemia[79], iron deficiency anemia[80], vitamin B12 deficiency anemia[81], could also impair cardiac autonomic function. Nevertheless, some evidence demonstrated that autonomic status determined by HRV is correlated with iron overload condition. In a study with thalassemic mice[82], it has been shown that

those thalassemic mice had a higher Lfnu, lower Hfnu, and higher Lf/Hf ratio than those in the wild-type mice. More interestingly, iron administration in both types of mice resulted in significantly higher NTBI levels concomitant with increased Lfnu and Lf/Hf ratio and decreased Hfnu. Moreover, iron chelator significantly decreased the Lfnu, Lf/Hf ratio, and increased the Hfnu in those iron overload thalassemic mice. This prospective study suggested that iron overload condition could contribute to progressive deterioration of the impaired cardiac autonomic function.

In conclusion, although CMR T2* is now recognized as the method of choice in evaluation of iron deposition in the heart[51], evidence suggested that TM patients must be prevented rather than treated even before cardiac iron loading becomes detectable on CMR T2* because of leading causes of cardiac tissue damage by other iron mediated mechanisms, such as those induced by labile plasma iron[83]. HRV might be used as an alternative approach to assess cardiac involvement in TM patients. Due to its easy access and much lower cost compared to CMR T2*, 24-h Holter monitoring for HRV analysis can be performed in most health providing centers. However, more evidence is needed to validate its use before it can be applied in clinical practice. Further studies are also needed to demonstrate the correlation between HRV and CMR T2* as well as the clinical application of HRV as a predictive marker in TM patients.

## REFERENCES


1 **Flint J**, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol* 1998; **11**: 1-51 [PMID: 10872472 DOI: 10.1016/S0950353(98)800693]

2 **Aydinok Y**. Thalassemia. *Hematology* 2012; **17** Suppl 1: S28-S31 [PMID: 22507773 DOI: 10.1179/102453312X13336169155295]

3 **Hershko C**. Pathogenesis and management of iron toxicity in thalassemia. *Ann N Y Acad Sci* 2010; **1202**: 1-9 [PMID: 20712765 DOI: 10.1111/j.17496632.2010.05544.x]

4 **Linn S**. DNA damage by iron and hydrogen peroxide in vitro and in vivo. *Drug Metab Rev* 1998; **30**: 313-326 [PMID: 9606606 DOI: 10.3109/03602539808996315]

5 **Borgna-Pignatti C**, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004; **89**: 1187-1193 [PMID: 15477202]

6 **Zareifar S**, Jabbari A, Cohan N, Haghpanah S. Efficacy of combined desferrioxamine and deferiprone versus single desferrioxamine therapy in patients with major thalassemia. *Arch Iran Med* 2009; **12**: 488-491 [PMID: 19722772]

7 **Taher A**, Al Jefri A, Elalfy MS, Al Zir K, Daar S, Rofail D, Baladi JF, Habr D, Kriemler-Krahn U, El-Beshlawy A. Improved treatment satisfaction and convenience with deferasirox in iron-overloaded patients with beta-Thalassemia: Results from the ESCALATOR Trial. *Acta Haematol* 2010; **123**: 220-225 [PMID: 20424435]

8 **Tanner MA**, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, Pibiri M, Nair SV, Walker JM, Pennell DJ. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. *J Cardiovasc Magn Reson* 2008; **10**: 12 [PMID: 18298856 DOI: 10.1186/1532429X1012]

9 **Davis BA**, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. *Blood* 2000; **95**: 1229-1236 [PMID: 10666195]

10 **Felker GM**, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, Baughman KL, Kasper EK. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med* 2000; **342**: 1077-1084 [PMID: 10760308 DOI: 10.1056/NEJM200004133421502]

11 **Olivieri NF**, Brittenham GM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, McClelland RA, Liu PP, Templeton DM, Koren G. Iron-chelation therapy with oral deferipronein patients with thalassemia major. *N Engl J Med* 1995; **332**: 918-922 [PMID: 7877649 DOI: 10.1056/NEJM199504063321404]

12 **Galanello R**, Piga A, Forni GL, Bertrand Y, Foschini ML, Bordone E, Leoni G, Lavagetto A, Zappu A, Longo F, Maseruka H, Hewson N, Sechaud R, Belleli R, Alberti D. Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in pediatric patients with beta-thalassemia major. *Haematologica* 2006; **91**: 1343-1351 [PMID: 17018383]

13 **Olivieri NF**, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood* 1997; **89**: 739-761 [PMID: 9028304]

14 **Piperno A**. Classification and diagnosis of iron overload. *Haematologica* 1998; **83**: 447-455 [PMID: 9658731]

15 **Orino K**, Lehman L, Tsuji Y, Ayaki H, Torti SV, Torti FM. Ferritin and the response to oxidative stress. *Biochem J* 2001; **357**: 241-247 [PMID: 11415455]

16 **Anderson LJ**, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001; **22**: 2171-2179 [PMID: 11913479 DOI: 10.1053/euhj.2001.2822]

17 **Anderson LJ**, Westwood MA, Holden S, Davis B, Prescott E, Wonke B, Porter JB, Walker JM, Pennell DJ. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol* 2004; **127**: 348-355 [PMID: 15491298 DOI: 10.1111/j.13652141.2004.05202.x]

18 **Noetzli LJ**, Carson SM, Nord AS, Coates TD, Wood JC. Longitudinal analysis of heart and liver iron in thalassemia major. *Blood* 2008; **112**: 2973-2978 [PMID: 18650452 DOI: 10.1182/blood200804148767]

19 **Kolnagou A**, Economides C, Eracleous E, Kontoghiorghes GJ. Low serum ferritin levels are misleading for detecting cardiac iron overload and increase the risk of cardiomyopathy in thalassemia patients. The importance of cardiac iron overload monitoring using magnetic resonance imaging T2 and T2*. *Hemoglobin* 2006; **30**: 219-227 [PMID: 16798647 DOI: 10.1080/03630260600642542]

20 **Rutjanaprom W**, Kanlop N, Charoenkwan P, Sittiwangkul R, Srichairatanakool S, Tantiworawit A, Phrommintikul A, Chattipakorn S, Fucharoen S, Chattipakorn N. Heart rate variability in beta-thalassemia patients. *Eur J Haematol* 2009; **83**: 483-489 [PMID: 19594617 DOI: 10.1111/j.16000609.2009.01314.x]

21 **Brili SV**, Tzonou AI, Castelanos SS, Aggeli CJ, Tentolouris CA, Pitsavos CE, Toutouzas PK. The effect of iron overload in the hearts of patients with beta-thalassemia. *Clin Cardiol* 1997; **20**: 541-546 [PMID: 9181265 DOI: 10.1002/clc.4960200607]

22 **Bayraktaroğlu S**, Aydinok Y, Yildiz D, Uluer H, Savaş R, Alper H. The relationship between the myocardial T2* value and left ventricular volumetric and functional pa-

rameters in thalassemia major patients. *Diagn Interv Radiol* 2011; **17**: 346-351 [PMID: 21647857 DOI: 10.4261/13053825.DIR.393310.2]
23 **Walker JM**, Nair S. Detection of the cardiovascular complications of thalassemia by echocardiography. *Ann N Y Acad Sci* 2010; **1202**: 165-172 [PMID: 20712789 DOI: 10.1111/j.17496632.2010.05643.x]
24 **Westwood MA**, Anderson LJ, Maceira AM, Shah FT, Prescott E, Porter JB, Wonke B, Walker JM, Pennell DJ. Normalized left ventricular volumes and function in thalassemia major patients with normal myocardial iron. *J Magn Reson Imaging* 2007; **25**: 1147-1151 [PMID: 17520718 DOI: 10.1002/jmri.20915]
25 **Hoffbrand AV**. Diagnosing myocardial iron overload. *Eur Heart J* 2001; **22**: 2140-2141 [PMID: 11913473 DOI: 10.1053/euhj.2001.2951]
26 **Kaye SB**, Owen M. Cardiac arrhythmias in thalassaemia major: evaluation of chelation treatment using ambulatory monitoring. *Br Med J* 1978; **1**: 342 [PMID: 623984 DOI: 10.1136/bmj.1.6109.342]
27 **Engle MA,** Erlandson M, Smith CH. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. *Circulation* 1964; **30**: 698-705 [PMID: 14226168 DOI: 10.1161/01.CIR.30.5.698]
28 **Kremastinos DT**, Tsetsos GA, Tsiapras DP, Karavolias GK, Ladis VA, Kattamis CA. Heart failure in beta thalassemia: a 5-year follow-up study. *Am J Med* 2001; **111**: 349-354 [PMID: 11583636 DOI: 10.1016/S00029343(01)008798]
29 **Cavallaro L**, Meo A, Busà G, Coglitore A, Sergi G, Satullo G, Donato A, Calabrò MP, Miceli M. [Arrhythmia in thalassemia major: evaluation of iron chelating therapy by dynamic ECG]. *Minerva Cardioangiol* 1993; **41**: 297-301 [PMID: 8233011]
30 **Ehlers KH**, Giardina PJ, Lesser ML, Engle MA, Hilgartner MW. Prolonged survival in patients with beta-thalassemia major treated with deferoxamine. *J Pediatr* 1991; **118**: 540-545 [PMID: 2007928 DOI: 10.1016/S00223476(05)833748]
31 **Qureshi N**, Avasarala K, Foote D, Vichinsky EP. Utility of Holter electrocardiogram in iron-overloaded hemoglobinopathies. *Ann N Y Acad Sci* 2005; **1054**: 476-480 [PMID: 16339701 DOI: 10.1196/annals.1345.064]
32 **Angelucci E**, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. *N Engl J Med* 2000; **343**: 327-331 [PMID: 10922422 DOI: 10.1056/NEJM200008033430503]
33 **Berdoukas V**, Dakin C, Freema A, Fraser I, Aessopos A, Bohane T. Lack of correlation between iron overload cardiac dysfunction and needle liver biopsy iron concentration. *Haematologica* 2005; **90**: 685-686 [PMID: 15921384]
34 **Fitchett DH**, Coltart DJ, Littler WA, Leyland MJ, Trueman T, Gozzard DI, Peters TJ. Cardiac involvement in secondary haemochromatosis: a catheter biopsy study and analysis of myocardium. *Cardiovasc Res* 1980; **14**: 719-724 [PMID: 7260965 DOI: 10.1093/cvr/14.12.719]
35 **Buja LM**, Roberts WC. Iron in the heart. Etiology and clinical significance. *Am J Med* 1971; **51**: 209-221 [PMID: 5095527 DOI: 10.1016/00029343(71)902403]
36 **Nielsen P**, Engelhardt R, Duerken M, Janka GE, Fischer R. Using SQUID biomagnetic liver susceptometry in the treatment of thalassemia and other iron loading diseases. *Transfus Sci* 2000; **23**: 257-258 [PMID: 11099909 DOI: 10.1016/S09553886(00)001016]
37 **Fischer R**, Longo F, Nielsen P, Engelhardt R, Hider RC, Piga A. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with beta-thalassaemia major: application of SQUID biomagnetic liver susceptometry. *Br J Haematol* 2003; **121**: 938-948 [PMID: 12786807]
38 **Sternickel K,** Braginski A. Biomagnetism using squids: Status and perspectives. *Supercond Sci Technol* 2006; **19** [DOI: 10.1088/09532048/19/3/024]
39 **Brissot P**, Ropert M, Le Lan C, Loréal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta* 2012; **1820**: 403-410 [PMID: 21855608 DOI: 10.1016/j.bbagen.2011.07.014]
40 **Anderson GJ**. Mechanisms of iron loading and toxicity. *Am J Hematol* 2007; **82**: 1128-1131 [PMID: 17963252 DOI: 10.1002/ajh.21075]
41 **Link G**, Pinson A, Hershko C. Heart cells in culture: a model of myocardial iron overload and chelation. *J Lab Clin Med* 1985; **106**: 147-153 [PMID: 4020242]
42 **Cabantchik ZI**, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. *Best Pract Res Clin Haematol* 2005; **18**: 277-287 [PMID: 15737890 DOI: 10.1016/j.beha.2004.10.003]
43 **Kaplan J**, Jordan I, Sturrock A. Regulation of the transferrin-independent iron transport system in cultured cells. *J Biol Chem* 1991; **266**: 2997-3004 [PMID: 1993673]
44 **Parkes JG**, Hussain RA, Olivieri NF, Templeton DM. Effects of iron loading on uptake, speciation, and chelation of iron in cultured myocardial cells. *J Lab Clin Med* 1993; **122**: 36-47 [PMID: 8320489]
45 **Piga A**, Longo F, Duca L, Roggero S, Vinciguerra T, Calabrese R, Hershko C, Cappellini MD. High nontransferrin bound iron levels and heart disease in thalassemia major. *Am J Hematol* 2009; **84**: 29-33 [PMID: 19006228 DOI: 10.1002/ajh.21317]
46 **al-Refaie FN**, Wickens DG, Wonke B, Kontoghiorghes GJ, Hoffbrand AV. Serum non-transferrin-bound iron in beta-thalassaemia major patients treated with desferrioxamine and L1. *Br J Haematol* 1992; **82**: 431-436 [PMID: 1419825]
47 **Jakeman A**, Thompson T, McHattie J, Lehotay DC. Sensitive method for nontransferrin-bound iron quantification by graphite furnace atomic absorption spectrometry. *Clin Biochem* 2001; **34**: 43-47 [PMID: 11239514 DOI: 10.1016/S00099120(00)001946]
48 **Hider RC**, Silva AM, Podinovskaia M, Ma Y. Monitoring the efficiency of iron chelation therapy: the potential of non-transferrin-bound iron. *Ann N Y Acad Sci* 2010; **1202**: 94-99 [PMID: 20712779 DOI: 10.1111/j.17496632.2010.05573.x]
49 **Jacobs EM**, Hendriks JC, van Tits BL, Evans PJ, Breuer W, Liu DY, Jansen EH, Jauhiainen K, Sturm B, Porter JB, Scheiber-Mojdehkar B, von Bonsdorff L, Cabantchik ZI, Hider RC, Swinkels DW. Results of an international round robin for the quantification of serum non-transferrin-bound iron: Need for defining standardization and a clinically relevant isoform. *Anal Biochem* 2005; **341**: 241-250 [PMID: 15907869 DOI: 10.1016/j.ab.2005.03.008]
50 **Pennell DJ**. T2* magnetic resonance and myocardial iron in thalassemia. *Ann N Y Acad Sci* 2005; **1054**: 373-378 [PMID: 16339685 DOI: 10.1196/annals.1345.045]
51 **Brittenham GM**. Iron-chelating therapy for transfusional iron overload. *N Engl J Med* 2011; **364**: 146-156 [PMID: 21226580 DOI: 10.1056/NEJMct1004810]
52 **Westwood MA**, Wonke B, Maceira AM, Prescott E, Walker JM, Porter JB, Pennell DJ. Left ventricular diastolic function compared with T2* cardiovascular magnetic resonance for early detection of myocardial iron overload in thalassemia major. *J Magn Reson Imaging* 2005; **22**: 229-233 [PMID: 16028255 DOI: 10.1002/jmri.20379]
53 **Ghugre NR**, Enriquez CM, Gonzalez I, Nelson MD, Coates TD, Wood JC. MRI detects myocardial iron in the human heart. *Magn Reson Med* 2006; **56**: 681-686 [PMID: 16888797 DOI: 10.1002/mrm.20981]
54 **Wood JC**, Otto-Duessel M, Aguilar M, Nick H, Nelson MD, Coates TD, Pollack H, Moats R. Cardiac iron determines cardiac T2*, T2, and T1 in the gerbil model of iron cardiomyopathy. *Circulation* 2005; **112**: 535-543 [PMID: 16027257 DOI: 10.1161/CIRCULATIONAHA.104.504415]

55 **Olson LJ**, Edwards WD, McCall JT, Ilstrup DM, Gersh BJ. Cardiac iron deposition in idiopathic hemochromatosis: histologic and analytic assessment of 14 hearts from autopsy. *J Am Coll Cardiol* 1987; **10**: 1239-1243 [PMID: 3680791]

56 **Kirk P**, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ, Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation* 2009; **120**: 1961-1968 [PMID: 19801505 DOI: 10.1161/CIRCULATIONAHA.109.874487]

57 **Merchant R**, Joshi A, Ahmed J, Krishnan P, Jankharia B. Evaluation of cardiac iron load by cardiac magnetic resonance in thalassemia. *Indian Pediatr* 2011; **48**: 697-701 [PMID: 21169646 DOI: 10.1007/s1331201101159]

58 **Kolnagou A**, Natsiopoulos K, Kleanthous M, Ioannou A, Kontoghiorghes GJ. Liver iron and serum ferritin levels are misleading for estimating cardiac, pancreatic, splenic and total body iron load in thalassemia patients: factors influencing the heterogenic distribution of excess storage iron in organs as identified by MRI T2*. *Toxicol Mech Methods* 2013; **23**: 48-56 [PMID: 22943064 DOI: 10.3109/15376516.2012.727198]

59 **Marsella M**, Borgna-Pignatti C, Meloni A, Caldarelli V, Dell'Amico MC, Spasiano A, Pitrolo L, Cracolici E, Valeri G, Positano V, Lombardi M, Pepe A. Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study. *Haematologica* 2011; **96**: 515-520 [PMID: 21228034 DOI: 10.3324/haematol.2010.025510]

60 **Tanner MA**, Galanello R, Dessi C, Westwood MA, Smith GC, Nair SV, Anderson LJ, Walker JM, Pennell DJ. Myocardial iron loading in patients with thalassemia major on deferoxamine chelation. *J Cardiovasc Magn Reson* 2006; **8**: 543-547 [PMID: 16755844 DOI: 10.1080/10976640600698155]

61 **Wood JC**, Tyszka JM, Carson S, Nelson MD, Coates TD. Myocardial iron loading in transfusion-dependent thalassemia and sickle cell disease. *Blood* 2004; **103**: 1934-1936 [PMID: 14630822 DOI: 10.1182/blood2003061919]

62 **Tanner M**, Porter J, Westwood M, Nair S, Anderson L, Walker J, Pennell D. Myocardial t2 in patients with cardiac failure secondary to iron overload. *Blood* 2005; **106**: 406 (Abstract)

63 **Modell B**, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2008; **10**: 42 [PMID: 18817553 DOI: 10.1186/1532429X1042]

64 **Chouliaras G**, Berdoukas V, Ladis V, Kattamis A, Chatziliami A, Fragodimitri C, Karabatsos F, Youssef J, Karagiorga-Lagana M. Impact of magnetic resonance imaging on cardiac mortality in thalassemia major. *J Magn Reson Imaging* 2011; **34**: 56-59 [PMID: 21608067 DOI: 10.1002/jmri.22621]

65 **Rajendra Acharya U**, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. *Med Biol Eng Comput* 2006; **44**: 1031-1051 [PMID: 17111118 DOI: 10.1007/s1151700601190]

66 **Chattipakorn N**, Incharoen T, Kanlop N, Chattipakorn S. Heart rate variability in myocardial infarction and heart failure. *Int J Cardiol* 2007; **120**: 289-296 [PMID: 17349699 DOI: 10.1016/j.ijcard.2006.11.221]

67 **Malik M**, Camm AJ, Janse MJ, Julian DG, Frangin GA, Schwartz PJ. Depressed heart rate variability identifies postinfarction patients who might benefit from prophylactic treatment with amiodarone: a substudy of EMIAT (The European Myocardial Infarct Amiodarone Trial). *J Am Coll Cardiol* 2000; **35**: 1263-1275 [PMID: 10758969 DOI: 10.1016/S07351097(00)005714]

68 **Kleiger RE**, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987; **59**: 256-262 [PMID: 3812275 DOI: 10.1016/00029149(87)907958]

69 **Zuanetti G**, Neilson JM, Latini R, Santoro E, Maggioni AP, Ewing DJ. Prognostic significance of heart rate variability in post-myocardial infarction patients in the fibrinolytic era. The GISSI-2 results. Gruppo Italiano per lo Studio della Sopravvivenza nell' Infarto Miocardico. *Circulation* 1996; **94**: 432-436 [PMID: 8759085 DOI: 10.1161/01.CIR.94.3.432]

70 **Stein PK**, Domitrovich PP, Huikuri HV, Kleiger RE. Traditional and nonlinear heart rate variability are each independently associated with mortality after myocardial infarction. *J Cardiovasc Electrophysiol* 2005; **16**: 13-20 [PMID: 15673380 DOI: 10.1046/j.15408167.2005.04358.x]

71 **Saul JP**, Arai Y, Berger RD, Lilly LS, Colucci WS, Cohen RJ. Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. *Am J Cardiol* 1988; **61**: 1292-1299 [PMID: 3376889 DOI: 10.1016/00029149(88)911721]

72 **Nolan J**, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, Baig W, Flapan AD, Cowley A, Prescott RJ, Neilson JM, Fox KA. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation* 1998; **98**: 1510-1516 [PMID: 9769304 DOI: 10.1161/01.CIR.98.15.1510]

73 **Havlicekova Z**, Jurko A. Heart rate variability changes in children after cardiac transplantation. *Bratisl Lek Listy* 2005; **106**: 168-170 [PMID: 16080362]

74 **Bellavere F**, Balzani I, De Masi G, Carraro M, Carenza P, Cobelli C, Thomaseth K. Power spectral analysis of heart-rate variations improves assessment of diabetic cardiac autonomic neuropathy. *Diabetes* 1992; **41**: 633-640 [PMID: 1568534 DOI: 10.2337/diab.41.5.633]

75 **Franzoni F**, Galetta F, Di Muro C, Buti G, Pentimone F, Santoro G. Heart rate variability and ventricular late potentials in beta-thalassemia major. *Haematologica* 2004; **89**: 233-234 [PMID: 15003899]

76 **Stamboulis E**, Vlachou N, Voumvourakis K, Andrikopoulou A, Arvaniti C, Tsivgoulis A, Athanasiadis D, Tsiodras S, Tentolouris N, Triantafyllidi H, Drossou-Servou M, Loutradi-Anagnostou A, Tsivgoulis G. Subclinical autonomic dysfunction in patients with β-thalassemia. *Clin Auton Res* 2012; **22**: 147-150 [PMID: 22170296 DOI: 10.1007/s1028601101542]

77 **Kardelen F**, Tezcan G, Akcurin G, Ertug H, Yesilipek A. Heart rate variability in patients with thalassemia major. *Pediatr Cardiol* 2008; **29**: 935-939 [PMID: 18551333 DOI: 10.1007/s0024600892401]

78 **De Chiara B**, Crivellaro W, Sara R, Ruffini L, Parolini M, Fesslovà V, Carnelli V, Fiorentini C, Parodi O. Early detection of cardiac dysfunction in thalassemic patients by radionuclide angiography and heart rate variability analysis. *Eur J Haematol* 2005; **74**: 517-522 [PMID: 15876256 DOI: 10.1111/j.16000609.2005.00434.x]

79 **Romero Mestre JC**, Hernández A, Agramonte O, Hernández P. Cardiovascular autonomic dysfunction in sickle cell anemia: a possible risk factor for sudden death? *Clin Auton Res* 1997; **7**: 121-125 [PMID: 9232355 DOI: 10.1007/BF02308838]

80 **Yokusoglu M**, Nevruz O, Baysan O, Uzun M, Demirkol S, Avcu F, Koz C, Cetin T, Hasimi A, Ural AU, Isik E. The altered autonomic nervous system activity in iron deficiency anemia. *Tohoku J Exp Med* 2007; **212**: 397-402 [PMID: 17660705 DOI: 10.1620/tjem.212.397]

81 **Sözen AB**, Demirel S, Akkaya V, Kudat H, Tükek T, Yeneral M, Ozcan M, Güven O, Korkut F. Autonomic dysfunction in vitamin B12 deficiency: a heart rate variability study. *J Auton Nerv Syst* 1998; **71**: 25-27 [PMID: 9722191 DOI: 10.1016/S01651838(98)000587]

82 **Kumfu S**, Chattipakorn S, Chinda K, Fucharoen S, Chattipakorn N. T-type calcium channel blockade improves survival and cardiovascular function in thalassemic mice. *Eur J Haematol* 2012; **88**: 535-548 [PMID: 22404220 DOI: 10.1111/

j.16000609.2012.01779.x]
83 **Di Tucci AA**, Matta G, Deplano S, Gabbas A, Depau C, Derudas D, Caocci G, Agus A, Angelucci E. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. *Haematologica* 2008; **93**: 1385-1388 [PMID: 18603557 DOI: 10.3324/haematol.12759]
84 **Barzin M**, Kowsarian M, Akhlaghpoor S, Jalalian R, Taremi M. Correlation of cardiac MRI T2* with echocardiography in thalassemia major. *Eur Rev Med Pharmacol Sci* 2012; **16**: 254-260 [PMID: 22428478]
85 **Brittenham GM**, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, Allen CJ, Farrell DE, Harris JW. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med* 1994; **331**: 567-573 [PMID: 8047080]
86 **Fischer R**, Tiemann CD, Engelhardt R, Nielsen P, Dürken M, Gabbe EE, Janka GE. Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptometry. *Am J Hematol* 1999; **60**: 289-299 [PMID: 10203103 DOI: 10.1002/(SICI)10968652(199904)]
87 **Hershko C**, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. *Br J Haematol* 1978; **40**: 255-263 [PMID: 708645]
88 **Oztarhan K**, Delibas Y, Salcioglu Z, Kaya G, Bakari S, Bornaun H, Aydogan G. Assessment of cardiac parameters in evaluation of cardiac functions in patients with thalassemia major. *Pediatr Hematol Oncol* 2012; **29**: 220-234 [PMID: 22475298 DOI: 10.3109/08880018.2012.671449]
89 **Gurses D**, Ulger Z, Levent E, Aydinok Y, Ozyurek AR. Time domain heart rate variability analysis in patients with thalassaemia major. *Acta Cardiol* 2005; **60**: 477-481 [PMID: 16261777 DOI: 10.2143/AC.60.5.2004967]
90 **Veglio F**, Melchio R, Rabbia F, Molino P, Genova GC, Martini G, Schiavone D, Piga A, Chiandussi L. Blood pressure and heart rate in young thalassemia major patients. *Am J Hypertens* 1998; **11**: 539-547 [PMID: 9633789 DOI: 10.1016/S08957061(97)00263X]
91 **Ma QL**, Wang B, Fu GH, Chen GF, Chen ZY. [Heart rate variability in children with beta-thalassemia major]. *Zhongguo Dangdai Erke Zazhi* 2011; **13**: 654-656 [PMID: 21849117]
92 **Incharoen T**, Thephinlap C, Srichairatanakool S, Chattipakorn S, Winichagoon P, Fucharoen S, Vadolas J, Chattipakorn N. Heart rate variability in beta-thalassemic mice. *Int J Cardiol* 2007; **121**: 203-204 [PMID: 17113168 DOI: 10.1016/j.ijcard.2006.08.076]
93 **Thephinlap C**, Phisalaphong C, Lailerd N, Chattipakorn N, Winichagoon P, Vadolus J, Fucharoen S, Porter JB, Srichairatanakool S. Reversal of cardiac iron loading and dysfunction in thalassemic mice by curcuminoids. *Med Chem* 2011; **7**: 62-69 [PMID: 21235521 DOI: 10.2174/157340611794072724]

**P- Reviewers** Aggarwal A, Aronow WS, Said S, Sun ZH
**S- Editor** Gou SX **L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.280

*World J Cardiol* 2013 August 26; 5(8): 280-287
ISSN 1949-8462 (online)


BRIEF ARTICLE

# Catheter ablation of atrial fibrillation: Radiofrequency catheter ablation for redo procedures after cryoablation

Klaus Kettering, Felix Gramley

Klaus Kettering, Felix Gramley, Department of Cardiology, University of Frankfurt, 60590 Frankfurt, Germany
Author contributions: Both authors have contributed significantly to this manuscript. They have made substantial contributions to the design of the study, to the acquisition of data and to the interpretation of the results. They have revised the manuscript critically for its scientific content. Both authors have approved the final version of the manuscript.
Correspondence to: Klaus Kettering, MD, Department of Cardiology, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany. klaus.kettering@t-online.de
Telephone: +49-69-63017273 Fax: +49-69-63016457
Received: February 25, 2013 Revised: June 20, 2013
Accepted: July 18, 2013
Published online: August 26, 2013

## Abstract

**AIM:** To evaluate the effectiveness of two different strategies using radiofrequency catheter ablation for redo procedures after cryoablation of atrial fibrillation.

**METHODS:** Thirty patients (paroxysmal atrial fibrillation: 22 patients, persistent atrial fibrillation: 8 patients) had to undergo a redo procedure after initially successful circumferential pulmonary vein (PV) isolation with the cryoballoon technique (Arctic Front Balloon, CryoCath Technologies/Medtronic). The redo ablation procedures were performed using a segmental approach or a circumferential ablation strategy (CARTO; Biosense Webster) depending on the intra-procedural findings. After discharge, patients were scheduled for repeated visits at the arrhythmia clinic. A 7-day Holter monitoring was performed at 3, 12 and 24 mo after the ablation procedure.

**RESULTS:** During the redo procedure, a mean number of 2.9 re-conducting pulmonary veins (SD ± 1.0 PVs) were detected (using a circular mapping catheter). In 20 patients, a segmental approach was sufficient to eliminate the residual pulmonary vein conduction because there were only a few recovered pulmonary vein fibres. In the remaining 10 patients, a circumferential ablation strategy was used because of a complete recovery of the PV-LA conduction. All recovered pulmonary veins could be isolated successfully again. At 2-year follow-up, 73.3% of all patients were free from an arrhythmia recurrence (22/30). There were no major complications.

**CONCLUSION:** In patients with an initial circumferential pulmonary vein isolation using the cryoballoon technique, a repeat ablation procedure can be performed safely and effectively using radiofrequency catheter ablation.



**Key words:** Atrial fibrillation; Catheter ablation; Cryoablation; Pulmonary veins; Supraventricular arrhythmias

**Core tip:** Cryoablation has been shown to be a safe technique for pulmonary vein isolation. However, the arrhythmia recurrence rate is high. Therefore, we have summarized our initial experience with two different strategies for redo procedures using radiofrequency catheter ablation. Thirty patients had to undergo a redo procedure after initially successful circumferential pulmonary vein isolation with the cryoballoon technique. The redo ablation procedures were performed using a segmental approach or a circumferential ablation strategy depending on the intra-procedural findings. All recovered pulmonary veins could be isolated successfully again. At 2-year follow-up, 73.3% of all patients were free from an arrhythmia recurrence.

Kettering K, Gramley F. Catheter ablation of atrial fibrillation: Radiofrequency catheter ablation for redo procedures after cryo-

ablation. *World J Cardiol* 2013; 5(8): 280-287 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/280.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.280

## INTRODUCTION

Catheter ablation has become the first line of therapy in patients with symptomatic, recurrent, drug-refractory atrial fibrillation (AF)[1-7]. Cryoablation has been shown to be a safe and effective technique for pulmonary vein (PV) isolation[1]. Although the acute success rates are high there is a significant arrhythmia recurrence rate after cryoablation during midterm follow-up[8-14]. According to a recently published study, catheter ablation with the cryoballoon technique resulted in maintenance of sinus rhythm in 74% of patients with paroxysmal atrial fibrillation and in 42 % of patients with persistent atrial fibrillation [median follow-up: 12 (7-16) mo[15-21]. Recovery of pulmonary vein conduction is one major reason for recurrences of atrial fibrillation. This is a crucial issue for cryoablation of AF because the cryoballoon is available with a rigid uniform design only (size: 23 or 28 mm; CryoCath Technologies, Quebec, Canada/Medtronic, Minneapolis, MN, United States). Taking into account the high degree of variability of the pulmonary vein anatomy it becomes clear that the contact between the balloon catheter and the pulmonary vein ostium cannot be equally good in all parts of the PV ostium. Therefore, insufficient tissue contact of the cryoballoon seems to be a key mechanism for recovery of initially successfully isolated pulmonary veins and AF recurrences in these patients during follow-up[22-26].

There are no established strategies for redo procedures after pulmonary vein isolation with the cryoballoon technique. From a theoretical point of view it seems to be reasonable to use the cryoablation technique for the redo procedure again because of its favourable lesion characteristics[1,22]. Such procedures can be performed either by using the cryoballoon technique again or by using a standard cryoablation catheter *(e.g.,* Freezor Max; Medtronic). The major concern with the cryoballoon technique for redo procedures is that there might be insufficient tissue contact in the same areas as during the initial procedure again. This might be a risk factor for further arrhythmia recurrences. Alternatively, repeat ablations can be performed with a segmental approach and a standard cryoablation catheter (*e.g.,* Freezor Max; Medtronic), However, a segmental approach using a cryoablation catheter is limited by the long duration of the cryoapplications required for achieving permanent lesions and by the fact that the position of the cryoablation catheter cannot be optimized during energy delivery.

Because of these limitations radiofrequency catheter ablation seems to be a promising approach. Therefore, the aim of our study was to analyse the data on pulmonary vein conduction recovery after pulmonary vein isolation with the cryoballoon technique and to evaluate two different strategies for redo procedures using radiofrequency catheter ablation.

Depending on the extent of pulmonary vein conduction recovery we performed either a segmental pulmonary vein re-isolation or an anatomically-based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach using radiofrequency energy application[19,20].

## MATERIALS AND METHODS

### *Patient population*

A total of 30 patients (21 men, 9 women; mean age 59.6 ± 10.0 years) with a recurrence of symptomatic atrial fibrillation after pulmonary vein isolation with the cryoballoon technique were enrolled in this study. Table 1 summarizes clinical characteristics of the patients enrolled in our study. A repeat procedure was planned because of recurrent episodes of paroxysmal atrial fibrillation in 23 patients or persistent AF in 7 patients. The redo procedures were performed at a mean interval of 12.5 ± 9.3 mo) after the initial ablation procedure. Prior to the initial ablation procedure paroxysmal AF had been present in 22 patients and persistent AF had been present in 8 patients. The initial ablation procedures had been performed at our University Hospital Center (between November 2007 and July 2009). The initial patient cohort consisted of 103 patients undergoing cryoablation as the primary procedure. Thus, the overall arrhythmia recurrence rate was 29.1% after the initial cryoablation procedure. For the initial procedure a cryoballoon device had been used in all patients who had to undergo a repeat ablation procedure (23 mm: 0 patients, 28 mm: 30 patients; CryoCath Technologies/Medtronic). In addition, a standard cryoablation catheter (Freezor Max 3; Medtronic) had been used in 4 patients. During the initial procedure a mean number of 11.6 ± 4.9 cryoapplications had been made using the cryoballoon device and a mean number of 4.0 ± 2.0 cryoapplications had been made in those patients in whom the standard cryoablation catheter (Freezor Max; Medtronic) had been used. At the end of the initial ablation procedure all pulmonary veins were isolated successfully. There were no major complications during or after the initial ablation procedure.

The repeat ablation procedures were performed at our University Hospital Center between March 2008 and November 2009. Inclusion criteria were (1) documented episodes of recurrent atrial fibrillation (≥ 30 s) after an initial ablation procedure with the cryoballoon technique (taking into account a blanking period of 3 mo after the initial ablation procedure); (2) severe symptoms despite antiarrhythmic drug therapy (including beta-blockers) or prior attemps of electrical cardioversion; (3) ability and willingness to give informed consent; and (4) age between 18 and 85 years. Patients were not accepted for catheter ablation if one of the following conditions was present: severe valvular heart disease or any other concomitant

**Table 1 Clinical data**

| Clinical data | Group A | Group B | Total | *P* value |
|---|---|---|---|---|
| Patients (men/women) | 20 (13/7) | 10 (8/2) | 30 (21/9) | 0.67 |
| Age (yr, mean ± SD) | 60.0 ± 10.7 | 58.9 ± 9.2 | 59.6 ± 10.0 | 0.81 |
| Cardiac disease | | | | 0.84 |
| None | 8 | 6 | 14 | |
| CAD | 3 | 1 | 4 | |
| DCM | 2 | 1 | 3 | |
| Valvular heart disease[1] | 5 | 1 | 6 | |
| Other | 2 | 1 | 3 | |
| Left ventricular ejection fraction mean (SD) | 54.7% (12.0%) | 53.8% (10.3%) | 54.4% (11.2%) | 0.86 |
| Previous cardiac surgery | 1 | 1 | 2 | 1.00 |
| Current antiarrhythmic drug therapy prior to the initial ablation procedure | | | | 0.94 |
| Class Ⅰc (*e.g.*, Flecainide, Propafenone) | 3 | 2 | 5 | |
| Class Ⅲ (*e.g.*, Amiodarone, Sotalol) | 6 | 1 | 7 | |
| Beta-Blocker in combination with a class Ⅰc or class Ⅲ antiarrhythmic drug | 4/2 | 3/1 | 7/3 | |
| Beta-Blocker | 2 | 1 | 3 | |
| Digitalis | 1 | 1 | 2 | |
| Other | 2 | 1 | 3 | |
| Current antiarrhythmic drug therapy prior to the repeat ablation procedure | | | | 0.57 |
| Class Ic (*e.g.*, Flecainide, Propafenone) | 2 | 1 | 3 | |
| Class Ⅲ (*e.g.*, Amiodarone, Sotalol) | 4 | 1 | 5 | |
| Beta-Blocker in combination with a class Ⅰc or class Ⅲ antiarrhythmic drug | 4/2 | 2/1 | 6/3 | |
| Beta-Blocker | 4 | 1 | 5 | |
| Digitalis | 2 | 0 | 2 | |
| Other | 2 | 4 | 6 | |

[1]Not requiring surgery. CAD: Coronary artery disease; DCM: Dilated cardiomyopathy (left ventricular ejection fraction < 40 %).

cardiac disease requiring surgery, severely impaired left ventricular function (left ventricular ejection fraction < 20%), left atrial diameter > 65 mm (parasternal long-axis view), left atrial thrombus, hyperthyroidism, severe renal insufficiency (creatinine ⩾ 3 mg/dL) or another severe concomitant illness.

### Cardiac imaging

A three-dimensional transesophageal echocardiography (3-D TEE) was performed in all patients prior to the ablation procedure (X7-2t, 7 MHz/IE 33; Philips Healthcare). The images were available throughout the ablation procedure. The 3-D TEE reconstructions provided an excellent overview over the individual left atrial morphology thereby facilitating the ablation procedure.

### Ablation procedure

AF ablation procedures were performed under conscious sedation at our institution. For the electrophysiological study, vascular access was obtained *via* both femoral veins and the left femoral artery. A 2500-U IV bolus of heparin was given shortly thereafter. First, a 6-F decapolar catheter (Bard, Electrophysiology Division, Lowell, MA, United States) was positioned within the coronary sinus (CS). Then, a single (or double) transseptal puncture was performed under fluoroscopic guidance. Immediately before the transseptal puncture, a 5-F catheter was placed in the ascending aorta to mark this area and to enhance the safety of the procedure. In some patients no transseptal puncture was necessary because of a patent foramen ovale or a residual defect of the atrial septum. Then, a pulmonary vein angiography was performed. After that, all four pulmonary veins were reevaluated during sinus rhythm and during CS pacing using a Lasso-catheter (2515, 7F; Biosense Webster, Diamond Bar, CA, United States). If atrial fibrillation was present at the beginning of the ablation procedure an electrical cardioversion was performed. The further strategy was based on the findings documented by the circular mapping catheter: if there were 1-3 pulmonary veins with recovered PV conduction we decided to perform a re-isolation of the recovered pulmonary veins using a segmental approach (group A). If there was reconnection of all four pulmonary veins, an anatomically-based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach was performed (group B)[20]. In addition, we classified the degree of PV reconnection as minor (PV spike visible on ⩽ 4 bipoles of the Lasso catheter) or major (PV potential visible on ⩾ 5 bipoles of the Lasso catheter).

Then, a standard irrigated-tip ablation catheter (7F; D-type, 3.5-mm-tip; Biosense Webster, Diamond Bar, CA, United States) or a CARTO-catheter (NAVI-STAR; 7F; D-type; 4-mm-tip; Biosense Webster) was positioned within the left atrium. After that, a second iv bolus of heparin was administered. During the procedure, the activated clotting time (ACT) was determined at regular intervals to ensure an adequate anticoagulation (ACT between 250 and 300 s).

Then, a segmental re-isolation of the pulmonary veins was performed in the patients assigned to group A using the above-mentioned irrigated-tip ablation catheter (43°;

25-35 W). Pulmonary vein ablation was performed during sinus rhythm and pacing from the coronary sinus. Pacing was performed from the distal CS during isolation of the left pulmonary veins and from the proximal CS during right PV ablation. If atrial fibrillation was present at the beginning of the ablation procedure or recurred during the procedure an electrical cardioversion was performed. Successful pulmonary vein isolation was assumed if one of the following criteria was met: complete disappearance of the pulmonary vein potential or appearance of a dissociated PV potential (circumferential mapping catheter).

In group B, a circumferential pulmonary vein ablation was performed in combination with a potential-guided segmental approach in order to achieve complete pulmonary vein isolation. Furthermore, a linear lesion was created at the roof of the left atrium in some patients with persistent atrial fibrillation. In addition, catheter ablation of the mitral isthmus was performed in selected cases.

First, a circumferential pulmonary vein ablation was performed targeting the both left-sided pulmonary veins [43°; 30 W (posterior wall) - 35 W (anterior wall)]. In addition, a Lasso-catheter was placed in the left superior or left inferior pulmonary vein. After completing the circumferential ablation line around the left-sided PVs, the left superior pulmonary vein and the left inferior pulmonary vein were reevaluated using the circular mapping catheter. If there was no complete PV isolation additional RF energy applications [43°; (25) -30 W] were applied using a segmental approach (during sinus rhythm/CS pacing or recurrent AF). If the isolation of the left-sided PVs was assumed to be complete the right-sided PVs were targeted in the same way. Then, a linear lesion at the LA roof was created in selected patients [43°; 30(-35) W]. In a few patients an additional mitral isthmus ablation was performed (if there was evidence for left atrial isthmus-dependent flutter [43°; 35(-40) W].

If atrial fibrillation was still present thereafter, an electrical cardioversion was performed. Then, all four pulmonary veins were reevaluated during sinus rhythm using the circumferential mapping catheter.

If necessary additional RF applications were performed using a segmental approach to achieve complete isolation of all four pulmonary veins. Then, the linear lesions at the LA roof were reevaluated during sinus rhythm. The ablation catheter was navigated back along the entire lesion to assess the presence of low-amplitude electrograms and the presence of double potentials or fractionated electrograms. If sharp high-amplitude electrograms were noted, additional RF applications were delivered at these sites in order to achieve a complete ablation line.

In addition, the linear lesions to the mitral annulus were reevaluated (anterior mitral isthmus line). The presence of bidirectional mitral isthmus conduction block was assumed if the following criteria were met: (1) presence of an unidirectional conduction block documented by activation mapping during pacing from the distal bipole of the CS catheter (placed far within the coronary sinus) or the left atrial appendage; (2) documentation of a similar conduction time during pacing from the anteroseptal mitral annulus (*via* the ablation catheter) *vs* the distal coronary sinus or the left atrial appendage; and (3) a conduction time > 150 ms in both directions.

In all patients (group A/B), a standard stomach tube (Flocare Nutrisoft M; Nurtica Healthcare, Châtel-St.Denis, Switzerland) or a special EP catheter (7 F; Osypka, Rheinfelden-Herten, Germany) had been introduced *via* a nasogastric route immediately before the ablation procedure in order to mark the esophagus. RF energy applications were avoided if there was a close anatomical relationship to the esophagus (or the power output was reduced as described previously[19]).

Finally, the completeness of the pulmonary vein isolation and of all linear lesions was reassessed after a waiting period of at least 20 min. Repeat selective pulmonary vein angiographies were performed of all targeted PVs. In addition, catheter ablation of the right atrial isthmus was performed in patients with inducible or clinically documented episodes of typical atrial flutter. The completeness of the right atrial isthmus lines was confirmed by differential pacing manoeuvres in all cases.

For the ablation procedure, a Bard EP system (LabSystem Pro, EP Recording System; Bard, Electrophysiology Division, Lowell, MA) and a Stockert RF generator (EP-shuttle; Stockert, Freiburg, Germany) were used. High-resolution x-ray imaging was provided by a Philips device (Philips Medical Systems, Best, The Netherlands).

### Follow-up

After hospital discharge, patients were seen regularly on an outpatient basis. One month after the procedure, a physical examination, a resting electrocardiogram (ECG) and a transthoracic echocardiogram were performed. The patients were questioned whether there was any evidence for an arrhythmia recurrence. In addition, a long-term ECG recording (24-h) was performed.

Three months after the ablation procedure, the patients were re-examined in the same way except for the fact that a 7-d Holter monitoring was performed and that each patient underwent a repeat three-dimensional transesophageal echocardiography to rule out a pulmonary vein stenosis. Then, the patients were seen at 3-mo intervals if asymptomatic. If there was an arrhythmia recurrence or other problems occurred, the further follow-up and future strategy (*e.g.*, electrical cardioversion, repeat ablation procedure) were planned on an individual basis.

Twelve months and 24 mo after the ablation procedure another 7-d Holter monitoring was performed. A blanking period of 3 mo was employed after ablation when evaluating the follow-up results. In addition, all patients were given a questionnaire 24 mo after the ablation procedure. The aim of this questionnaire was to evaluate the clinical status of the patients and to reveal whether there was any evidence for arrhythmia recurrences not detected by the long-term ECG recordings[20].

Oral anticoagulation was continued for at least 3 mo

after the procedure in all patients. During the first three mo after catheter ablation the patients received the same antiarrhythmic medication as prior to the ablation procedure. If there was no evidence for an arrhythmia recurrence all antiarrhythmic drugs were discontinued thereafter except for beta-blockers. The beta-blocker therapy was continued thereafter in order to reduce the risk of arrhythmia recurrences during long-term follow-up and to achieve an adequate rate control if such arrhythmia recurrences occurred.

### Statistical analysis

All parameters with a normal distribution are given as mean (± 1 SD). All other parameters are presented as median and $25^{th}/75^{th}$ percentiles. $\chi^2$ tests, *t*-tests, and Fischer's exact test were used to compare nominal, continuous, and dichotomous characteristics of the two study groups at baseline as well as during follow-up. Significance was accepted if the *P* value was $< 0.05$. The statistical package of JMP (Version 3.2.6, SAS Institute, Cary, NC, United States) was used for data analysis.

## RESULTS

Thirty patients were enrolled in this study between March 2008 and November 2009. All of them had to undergo a repeat ablation procedure because of a recurrence of symptomatic atrial fibrillation after pulmonary vein isolation with the cryoballoon technique. Prior to the redo procedure, 23 patients suffered from recurrent episodes of paroxysmal atrial fibrillation and 7 patients suffered from persistent AF. The repeat ablation procedure could be performed as planned in all patients.

### Procedural results

**Evaluation of the pulmonary veins:** During the repeat procedure, a mean number of 2.9 ± 1.0 PVs with recovered PV conduction were detected (using a circular mapping catheter). In all patients at least one pulmonary vein with recovered PV conduction was observed. In 4 patients, there was only one pulmonary vein with recovered PV conduction. There were 6 patients with two reconnected veins and 10 patients with three reconnected pulmonary veins. In 10 patients, all four pulmonary veins showed recovered PV conduction. Seven out of 10 patients with four reconnected pulmonary veins suffered from persistent AF.

Minor PV reconnection (PV spike visible on 4 ⩽ bipoles of the Lasso catheter) was present in 77 out of 86 reconnected veins (89.5%). Major PV reconnection (PV spike visible on > 4 bipoles of the Lasso catheter) was found in 9 out of 86 reconnected PVs (10.5%).

### Ablation strategy

After evaluating the pulmonary veins the further ablation strategy was planned based on the intraprocedural findings. In 20 patients (with 1-3 reconnected PVs) re-isolation of the recovered pulmonary veins was performed using a segmental approach (group A). In 10 patients, all four pulmonary veins showed recovered PV conduction. In these patients, an anatomically-based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach was performed (group B). In 3 out of 10 patients in group B, an additional linear lesion was created at the LA roof. In 2 patients in group B, catheter ablation of the mitral isthmus was performed. In addition, catheter ablation of the right atrial isthmus was performed in 5 patients in group A and in 2 patients in group B ($P = 1.0$).

The ablation procedure could be performed as planned in all patients. The mean procedure time was 156 ± 41 min; group A: 147 ± 32 min; group B: 175 ± 58 min; $P = 0.07$. This included all preparations and a waiting period (20 min) at the end of the procedure for a final reevaluation of the completeness of the pulmonary vein isolation/linear lesions. The mean fluoroscopy dosage was 2520 ± 2055 cGycm²; group A: 2556 ± 2178 cGycm²; group B: 2450 ± 1810 cGycm²; $P = 0.87$.

The segmental approach could be performed successfully in all patients in group A. A mean number of 2.3 ± 0.8 PVs were re-isolated per patient. At the end of the procedure the complete isolation of all four pulmonary veins could be documented in all patients using a circumferential mapping catheter.

In group B, an anatomically-based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach was performed successfully in all patients. In this group, all four pulmonary veins could be re-isolated successfully in all patients (documented using a circular mapping catheter).

There were no major complications (*e.g.,* cardiac tamponade, transient ischemic attacks (TIAs) or stroke, significant pulmonary vein stenosis (⩾ 70%), periprocedural death) during the procedure in both groups. A transseptal puncture had to be performed in 17 out of 30 patients. In the other patients no transseptal puncture was necessary because of a patent foramen ovale (7 patients) or a residual defect of the atrial septum (6 patients).

### Clinical outcome

The mean follow-up was 1004 ± 751 d in group A and 821 ± 435 d in group B ($P = 0.53$). The mean overall follow-up was 940 ± 653 d. Six months after the redo procedure, 85.0% of the patients in group A (17/20) and 80.0% of the patients in group B (8/10) were free from an arrhythmia recurrence [$P = 1.0$; in total: 25/30 patients (83.3%)]. Twelve month after the repeat ablation procedure, 80.0% of all patients in group A (16/20) were still free from an arrhythmia recurrence compared to 70.0% of patients in group B (7/10; $P = 0.66$). Thus, the overall success rate was 76.7% at 1-year follow-up (no arrhythmia recurrence in 23 out of 30 patients). Two years after the redo procedure, the overall success rate was 73.3% (no arrhythmia recurrence in 22 out of 30 patients). Fifteen out of twenty patients in group A (75.0%) and 7 out of 10 patients in group B (70.0%) were still free from an ar-

rhythmia recurrence ($P = 1.0$).

According to the analysis of the questionnaire, 24/30 patients (80.0%) were completely asymptomatic at 24-mo follow-up. There were no major complications during or after the ablation procedures (including a follow-up duration of 24 mo). Minor complications were observed in 2 patients (pulmonary vein stenosis < 70%: 2 patients).

Analysing the clinical course of the patients who experienced an arrhythmia recurrence during follow-up, 7-d Holter monitoring revealed paroxysmal atrial fibrillation in 5 patients and persistent atrial fibrillation in 3 patients. No modification of the antiarrhythmic medication and no repeat ablation procedure was required in 2 patients with an arrhythmia recurrence because they were almost asymptomatic. In 2 patients with a symptomatic arrhythmia recurrence symptoms could be controlled by modifying the antiarrhythmic drug therapy. Four patients with symptomatic arrhythmia recurrences had to undergo a third ablation procedure.

## DISCUSSION

Catheter ablation has become an important therapeutic option for patients with highly symptomatic and drug-refractory atrial fibrillation. Cryoablation is a safe and effective technique for pulmonary vein isolation[1,21], which is the cornerstone of catheter ablation in patients with paroxysmal or persistent atrial fibrillation. However, there is a significant arrhythmia recurrence rate after cryoablation during midterm follow-up. Catheter ablation with the cryoballoon technique was reported to result in maintenance of sinus rhythm in 74% of patients with paroxysmal AF and in only 42% of patients with persistent AF during a median follow-up of 12 (7-16) mo[21]. Therefore, strategies for redo procedures are of major importance. However, there are no established strategies for redo procedures after PV isolation with the cryoballoon technique so far. Currently applied strategies for redo procedures are repeat ablation procedures with the cryoballoon technique and a segmental approach using a standard cryoablation catheter. Repeat ablation procedures with the cryoballoon technique are limited by the fact that the rigid design of the cryoballoon might result in insufficient tissue contact in the same areas during both procedures (thereby triggering further arrhythmia recurrences). Repeat ablation procedures with a standard cryoablation catheter and a segmental approach are mainly limited by the long duration of (repeated) cryoapplications required for creating permanent lesions.

Therefore, we have evaluated two different strategies for redo procedures using radiofrequency catheter ablation. These ablation strategies included either a mere segmental pulmonary vein re-isolation or alternatively an anatomically-based circumferential PV ablation in combination with a potential-guided segmental approach[19,20]. The decision about the ablation strategy was based on the extent of pulmonary vein conduction recovery (documented using a circular mapping catheter at the beginning of the redo procedure).

### Main results

During the redo procedures, a mean number of 2.9 re-conducting PVs (SD ± 1.0 PVs) were detected. In 20 patients, 1-3 re-conducting PVs were detected. There were only 10 patients in whom all four pulmonary veins showed recovered PV conduction. Minor PV reconnection (PV spike visible on 4 ⩽ bipoles of the Lasso catheter) was present in 77 out of 86 reconnected veins (89.5%). Major PV reconnection (PV spike visible on > 4 bipoles of the Lasso catheter) was found in 9 out of 86 reconnected PVs (10.5%).

In 20 patients (with 1-3 reconnected PVs) re-isolation of the recovered pulmonary veins was performed using a segmental approach (group A). An anatomically-based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach was performed in 10 patients, because recovered PV conduction of all four pulmonary veins was detected in these patients (group B).

Two years after the repeat ablation procedure, 75.0% of all patients in group A (15/20) were still free from an arrhythmia recurrence compared to 70.0% of patients in group B (7/10; $P = 1.0$). The overall success rate was 73.3% at 2-year follow-up. There were no major complications during or after the ablation procedures in both groups.

The results of our study demonstrate that a repeat ablation procedure after initial PV isolation using the cryoballoon technique can be performed safely and effectively using radiofrequency catheter ablation. In most cases only a few re-conducting PV fibres were found and therefore, a segmental re-ablation approach seems to be sufficient in the majority of patients.

There are two major advantages of radiofrequency catheter ablation over cryoablation for redo procedures after pulmonary vein isolation with the cryoballoon technique. First, due to the high degree of variability of the PV anatomy the contact between the cryoballoon catheter and the pulmonary veins cannot be equally good among all parts of the PV ostium. This limitation can be overcome during the redo procedure in most cases because the majority of areas with insufficient tissue contact during the initial procedure with the cryoballon technique can be easily reached with a standard RF ablation catheter.

Second, the use of radiofrequency energy delivery after prior cryoablation might result in a very stable lesion formation. Although there are no larger studies analysing the histological characteristics in this setting this effect might have contributed to the favourable results of our study. Nevertheless, further studies are necessary to evaluate the histological changes after repeated cryoablation in comparison to lesions created using RF ablation for redo procedures after cryoablation.

### Limitations

This is a single centre study and, therefore, it is of mod-

erate size. However, follow-up was meticulous including repeat three-dimensional transesophageal echocardiography 3 mo after the procedure to rule out a pulmonary vein stenosis. Furthermore, a 7-d Holter monitoring was performed twelve months and twenty-four months after the ablation procedure. The follow-up duration is longer than in many other studies and all patients underwent the final evaluation 2 years after the redo ablation procedure. Therefore, this study provides very reliable information about the long-term outcome of this patient cohort.

Obviously, the extent of pulmonary vein conduction recovery after cryoablation should be evaluated in a larger patient cohort in a future study. A large randomized study is needed to compare the effectiveness of different strategies for redo procedures after initial pulmonary vein isolation with the cryoballoon technique (*i.e.*, repeat ablation with the cryoballoon technique or a segmental approach using a standard cryoablation catheter, a segmental pulmonary vein re-isolation using a standard irrigated-tip RF ablation catheter and a circumferential ablation strategy using radiofrequency catheter ablation).

In conclusion, a repeat ablation procedure after initial pulmonary vein isolation using the cryoballoon technique can be performed safely and effectively using radiofrequency catheter ablation. In most cases only a few re-conducting PV fibres were found and therefore, a segmental re-ablation approach seems to be sufficient in the majority of patients. Alternatively, a circumferential approach can be performed using RF catheter ablation in patients with complete recovery of all four pulmonary veins. Obviously, the results of this study have to be confirmed in a larger randomized trial.

## COMMENTS

### Background

Catheter ablation has become the first line of therapy in patients with symptomatic, recurrent, drug-refractory atrial fibrillation. Cryoablation has been shown to be a safe and effective technique for pulmonary vein isolation.

### Research frontiers

However, the arrhythmia recurrence rate is high after cryoablation procedures and there are no established strategies for redo procedures in these patients. It is a matter of discussion whether cryoablation should be used for the redo procedures in these patients again or whether radiofrequency catheter ablation might be advantageous.

### Innovations and breakthroughs

We have summarized our initial experience with two different strategies for redo procedures after cryoablation of atrial fibrillation using radiofrequency catheter ablation. The redo ablation procedures were performed using a segmental approach or a circumferential ablation strategy depending on the intra-procedural findings (1-3 versus 4 reconnected pulmonary veins). In 20 patients, a segmental approach was sufficient to eliminate the residual pulmonary vein conduction because there were only a few recovered pulmonary vein fibres. In the remaining 10 patients, a circumferential ablation strategy was used because of a complete recovery of the PV-LA conduction. At 2-year follow-up, 73.3 % of all patients were free from an arrhythmia recurrence. The results demonstrate that a repeat ablation procedure after initial circumferential pulmonary vein isolation using the cryoballoon technique can be performed safely and effectively using radiofrequncy catheter ablation.

### Applications

The results suggest that radiofrequency catheter ablation is a good therapeutic option for the treatment of recurrences of atrial fibrillation after circumferential pulmonary vein isolation using the cryoballoon technique.

### Terminology

Catheter ablation: interventional technique for the treatment of cardiac arrhythmias. Atrial fibrillation: disorganized atrial arrhythmia which is mostly induced by ectopic beats originating from the pulmonary veins.

### Peer review

Well written, interesting review experience of the authors about the challenges and difficulties of redo-atrial fibrillation ablation.

## REFERENCES


1 **Kettering K**, Al-Ghobainy R, Wehrmann M, Vonthein R, Mewis C. Atrial linear lesions: feasibility using cryoablation. *Pacing Clin Electrophysiol* 2006; **29**: 283-289 [PMID: 16606396 DOI: 10.1111/j.1540-8159.2006.00335.x]

2 **Oral H**, Knight BP, Ozaydin M, Chugh A, Lai SW, Scharf C, Hassan S, Greenstein R, Han JD, Pelosi F, Strickberger SA, Morady F. Segmental ostial ablation to isolate the pulmonary veins during atrial fibrillation: feasibility and mechanistic insights. *Circulation* 2002; **106**: 1256-1262 [PMID: 12208802 DOI: 10.1161/01.CIR.0000027821.55835.00]

3 **Haïssaguerre M**, Shah DC, Jaïs P, Hocini M, Yamane T, Deisenhofer I, Garrigue S, Clémenty J. Mapping-guided ablation of pulmonary veins to cure atrial fibrillation. *Am J Cardiol* 2000; **86**: 9K-19K [PMID: 11084094 DOI: 10.1016/S0002-9149(00)01186-3]

4 **Gerstenfeld EP**, Guerra P, Sparks PB, Hattori K, Lesh MD. Clinical outcome after radiofrequency catheter ablation of focal atrial fibrillation triggers. *J Cardiovasc Electrophysiol* 2001; **12**: 900-908 [PMID: 11513440 DOI: 10.1046/j.1540-8167.2001.00900.x]

5 **Marrouche NF**, Dresing T, Cole C, Bash D, Saad E, Balaban K, Pavia SV, Schweikert R, Saliba W, Abdul-Karim A, Pisano E, Fanelli R, Tchou P, Natale A. Circular mapping and ablation of the pulmonary vein for treatment of atrial fibrillation: impact of different catheter technologies. *J Am Coll Cardiol* 2002; **40**: 464-474 [PMID: 12142112 DOI: 10.1016/S0735-1097(02)01972-1]

6 **Swartz JF**, Pellersels G, Silvers J, Patten L, Cervantez D. A catheter-based curative approach to atrial fibrillation in humans. *Circulation* 1994; **90**: I-335

7 **Haïssaguerre M**, Jaïs P, Shah DC, Gencel L, Pradeau V, Garrigues S, Chouairi S, Hocini M, Le Métayer P, Roudaut R, Clémenty J. Right and left atrial radiofrequency catheter therapy of paroxysmal atrial fibrillation. *J Cardiovasc Electrophysiol* 1996; **7**: 1132-1144 [PMID: 8985802 DOI: 10.1111/j.1540-8167.1996.tb00492.x]

8 **Ernst S**, Schlüter M, Ouyang F, Khanedani A, Cappato R, Hebe J, Volkmer M, Antz M, Kuck KH. Modification of the substrate for maintenance of idiopathic human atrial fibrillation: efficacy of radiofrequency ablation using nonfluoroscopic catheter guidance. *Circulation* 1999; **100**: 2085-2092 [PMID: 10562265 DOI: 10.1161/01.CIR.100.20.2085]

9 **Jaïs P**, Hocini M, Hsu LF, Sanders P, Scavee C, Weerasooriya R, Macle L, Raybaud F, Garrigue S, Shah DC, Le Metayer P, Clémenty J, Haïssaguerre M. Technique and results of linear ablation at the mitral isthmus. *Circulation* 2004; **110**: 2996-3002 [PMID: 15520313 DOI: 10.1161/01.CIR.0000146917.75041.58]

10 **Oral H**, Chugh A, Lemola K, Cheung P, Hall B, Good E, Han J, Tamirisa K, Bogun F, Pelosi F, Morady F. Noninducibility of atrial fibrillation as an end point of left atrial circumferential ablation for paroxysmal atrial fibrillation: a randomized study. *Circulation* 2004; **110**: 2797-2801 [PMID: 15505091 DOI: 10.1161/01.CIR.0000146786.87037.26]

11 **Avitall B**, Helms RW, Koblish JB, Sieben W, Kotov AV, Gupta GN. The creation of linear contiguous lesions in the atria with an expandable loop catheter. *J Am Coll Cardiol* 1999; **33**: 972-984 [PMID: 10091824 DOI: 10.1016/S0735-

1097(98)00686-X]
12 **Mitchell MA**, McRury ID, Haines DE. Linear atrial ablations in a canine model of chronic atrial fibrillation: morphological and electrophysiological observations. *Circulation* 1998; **97**: 1176-1185 [PMID: 9537344 DOI: 10.1161/01.CIR.97.12.1176]
13 **Schwartzman D**, Kuck KH. Anatomy-guided linear atrial lesions for radiofrequency catheter ablation of atrial fibrillation. *Pacing Clin Electrophysiol* 1998; **21**: 1959-1978 [PMID: 9793093 DOI: 10.1111/j.1540-8159.1998.tb00016.x]
14 **Ouyang F**, Bänsch D, Ernst S, Schaumann A, Hachiya H, Chen M, Chun J, Falk P, Khanedani A, Antz M, Kuck KH. Complete isolation of left atrium surrounding the pulmonary veins: new insights from the double-Lasso technique in paroxysmal atrial fibrillation. *Circulation* 2004; **110**: 2090-2096 [PMID: 15466640 DOI: 10.1161/01.CIR.0000144459.37455.EE]
15 **Ouyang F**, Antz M, Ernst S, Hachiya H, Mavrakis H, Deger FT, Schaumann A, Chun J, Falk P, Hennig D, Liu X, Bänsch D, Kuck KH. Recovered pulmonary vein conduction as a dominant factor for recurrent atrial tachyarrhythmias after complete circular isolation of the pulmonary veins: lessons from double Lasso technique. *Circulation* 2005; **111**: 127-135 [PMID: 15623542 DOI: 10.1161/01.CIR.0000151289.73085.36]
16 **Ouyang F**, Ernst S, Chun J, Bänsch D, Li Y, Schaumann A, Mavrakis H, Liu X, Deger FT, Schmidt B, Xue Y, Cao J, Hennig D, Huang H, Kuck KH, Antz M. Electrophysiological findings during ablation of persistent atrial fibrillation with electroanatomic mapping and double Lasso catheter technique. *Circulation* 2005; **112**: 3038-3048 [PMID: 16275866 DOI: 10.1161/CIRCULATIONAHA.105.561183]
17 **Kettering K**, Greil GF, Fenchel M, Kramer U, Weig HJ, Busch M, Miller S, Sieverding L, Laszlo R, Schreieck J. Catheter ablation of atrial fibrillation using the Navx-/Ensite-system and a CT-/MRI-guided approach. *Clin Res Cardiol* 2009; **98**: 285-296 [PMID: 19283334 DOI: 10.1007/s00392-009-0001-9]
18 **Kettering K**, Greil GF, Busch M, Miller S, Sieverding L, Schreieck J. Catheter ablation of atrial fibrillation: ongoing atrial fibrillation inside a single pulmonary vein after successful electrical disconnection and restoration of sinus rhythm in both atria. *Clin Res Cardiol* 2006; **95**: 663-667 [PMID: 16998744]
19 **Kettering K**, Weig HJ, Busch M, Laszlo R, Schreieck J. Segmental pulmonary vein ablation: success rates with and without exclusion of areas adjacent to the esophagus. *Pacing Clin Electrophysiol* 2008; **31**: 652-659 [PMID: 18507536 DOI: 10.1111/j.1540-8159.2008.01067.x]
20 **Kettering K**, Weig HJ, Busch M, Schneider KM, Eick C, Weretka S, Laszlo R, Gawaz M, Schreieck J. Catheter ablation of persistent atrial fibrillation: anatomically based circumferential pulmonary vein ablation in combination with a potential-guided segmental approach to achieve complete pulmonary vein isolation. *J Interv Card Electrophysiol* 2011; **30**: 63-72 [PMID: 21253841 DOI: 10.1007/s10840-010-9533-1]
21 **Neumann T**, Vogt J, Schumacher B, Dorszewski A, Kuniss M, Neuser H, Kurzidim K, Berkowitsch A, Koller M, Heintze J, Scholz U, Wetzel U, Schneider MA, Horstkotte D, Hamm CW, Pitschner HF. Circumferential pulmonary vein isolation with the cryoballoon technique results from a prospective 3-center study. *J Am Coll Cardiol* 2008; **52**: 273-278 [PMID: 18634982 DOI: 10.1016/j.jacc.2008.04.021]
22 **Lustgarten DL**, Keane D, Ruskin J. Cryothermal ablation: mechanism of tissue injury and current experience in the treatment of tachyarrhythmias. *Prog Cardiovasc Dis* 1999; **41**: 481-498 [PMID: 10445872]
23 **Chierchia GB**, Namdar M, Sarkozy A, Sorgente A, de Asmundis C, Casado-Arroyo R, Capulzini L, Bayrak F, Rodriguez-Mañero M, Ricciardi D, Rao JY, Overeinder I, Paparella G, Brugada P. Verification of pulmonary vein isolation during single transseptal cryoballoon ablation: a comparison between the classical circular mapping catheter and the inner lumen mapping catheter. *Europace* 2012; **14**: 1708-1714 [PMID: 22772051 DOI: 10.1093/europace/eus189]
24 **Schmidt M**, Dorwarth U, Straube F, Wankerl M, Krieg J, Leber AW, Ebersberger HU, Daccarett M, Huber A, Rummeny E, Hoffmann E. A novel double cryoballoon strategy in persistent atrial fibrillation: a pilot study. *Clin Res Cardiol* 2012; **101**: 777-785 [PMID: 22484346]
25 **Calkins H**, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen SA, Crijns HJ, Damiano RJ, Davies DW, DiMarco J, Edgerton J, Ellenbogen K, Ezekowitz MD, Haines DE, Haissaguerre M, Hindricks G, Iesaka Y, Jackman W, Jalife J, Jais P, Kalman J, Keane D, Kim YH, Kirchhof P, Klein G, Kottkamp H, Kumagai K, Lindsay BD, Mansour M, Marchlinski FE, McCarthy PM, Mont JL, Morady F, Nademanee K, Nakagawa H, Natale A, Nattel S, Packer DL, Pappone C, Prystowsky E, Raviele A, Reddy V, Ruskin JN, Shemin RJ, Tsao HM, Wilber D. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design. *Europace* 2012; **14**: 528-606 [PMID: 22389422 DOI: 10.1093/europace/eus027]
26 **Camm AJ,** Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, Hindricks G, Kirchhof P. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation--developed with the special contribution of the European Heart Rhythm Association. *Europace* 2012; **14**: 1385-1413 [PMID: 22923145 DOI: 10.1093/eurheartj/ehs253]

**P- Reviewers** Castillo R, CelikyurtY, MiyasakaY, Serebruany V
**S- Editor** Zhai HH **L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.288

*World J Cardiol* 2013 August 26; 5(8): 288-294
ISSN 1949-8462 (online)


BRIEF ARTICLE

# Patients with cardiac disease: Changes observed through last decade in out-patient clinics

Alberto Cordero, Vicente Bertomeu-Martínez, Pilar Mazón, Lorenzo Fácila, Juan Cosín, Vicente Bertomeu-González, Moisés Rodriguez, Eva Andrés, Enrique Galve, Iñaki Lekuona, Jose R González-Juanatey

Alberto Cordero, Vicente Bertomeu-Martínez, Pilar Mazón, Vicente Bertomeu-González, Cardiology Department, Hospital Universitario de San Juan, 03550 Alicante, Spain
Lorenzo Fácila, Cardiology Department, Hospital General de Valencia, 46014 Valencia, Spain
Juan Cosín, Cardiology Department, Hospital Arnau de Vilanova, 46015 Valencia, Spain
Moisés Rodriguez, Jose R González-Juanatey, Cardiology Department, Hospital Complejo Universitario de Santiago de Compostela, 15706 Santiago de Compostela, Spain
Eva Andrés, Research Unit, Hospital Universitario 12 de Octubre, Instituto de investigación 12 de Octubre (imas12), Ciber de Epidemiología y Salud Pública, 28080 Madrid, Spain
Eva Andrés, Cardiology Department, Clínica Universitaria de Navarra, 31008 Pamplona, Spain
Enrique Galve, Cardiology Department, Hospital Valle Hebrón, 08035 Barcelona, Spain
Iñaki Lekuona, Cardiology Department, Hospital Galdakano, 48960 Bilbao, Spain
Author contributions: Cordero A, Bertomeu-Martínez V and González-Juanatey JR designed research; Cordero A and Mazón P performed research; Andrés E and Rodriguez M contributed new reagents or analytic tools; Cordero A, Fácila L, Andrés E and Rodriguez M analyzed data; Cordero A, Bertomeu-Martínez V, Galve E, Lekuona I and González-Juanatey JR wrote the paper; all authors contributed to this paper.
Supported by An unrestricted grant of RECORDATI Laboratories, Spain
Correspondence to: Alberto Cordero, MD, PhD, FESC, Cardiology Department, Hospital Universitario de San Juan, Carretera Valencia-Alicante sn, San Juan de Alicante, 03550 Alicante, Spain. acorderofort@gmail.com
Telephone: +34-9659-38783 Fax: +34-96-5938652
Received: March 18, 2013 Revised: May 5, 2013
Accepted: August 4, 2013
Published online: August 26, 2013

## Abstract

**AIM:** To describe current profile of patients with cardiovascular disease (CVD) and assessing changes through last decade.

**METHODS:** Comparison of patients with established CVD from two similar cross-sectional registries performed in 1999 ($n$ = 6194) and 2009 ($n$ = 4639). The types of CVD were coronary heart disease (CHD), heart failure (HF) and atrial fibrillation (AF). Patients were collected from outpatient clinics. Investigators were 80% cardiologist and 20% primary care practitioners. Clinical antecedents, major diagnosis, blood test results and medical treatments were collected from all patients.

**RESULTS:** An increase in all risk factors, except for smoking, was observed; a 54.4% relative increase in BP control was noted. CHD was the most prevalent CVD but HF and AF increased significantly, 41.5% and 33.7%, respectively. A significant reduction in serum lipid levels was observed. The use of statins increased by 141.1% as did all cardiovascular treatments. Moreover, the use of angiotensin-renin system inhibitors in patients with HF, beta-blockers in CHD patients or oral anticoagulants in AF patients increased by 83.0%, 80.3% and 156.0%, respectively ($P$ < 0.01).

**CONCLUSION:** The prevalence of all cardiovascular risk factors has increased in patients with CVD through last decade. HF and AF have experienced the largest increases.



**Key words:** Cardiovascular disease; Trends; Heart failure; Coronary heart disease; Atrial fibrillation

**Core tip:** Reduction in acute phase of cardiovascular disease (CVD) has lead to a progressive increase in patients with chronic CVD that are considered high-risk patients and mostly attended in outpatient clinics.

The prevalence of all cardiovascular risk factors has increased in patients with CVD through last decade. Heart failure and atrial fibrillation have experienced the largest increases.

Cordero A, Bertomeu-Martínez V, Mazón P, Fácila L, Cosín J, Bertomeu-González V, Rodriguez M, Andrés E, Galve E, Lekuona I, González-Juanatey JR. Patients with cardiac disease: Changes observed through last decade in out-patient clinics. *World J Cardiol* 2013; 5(8): 288-294 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/288.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.288

## INTRODUCTION

Cardiovascular disease (CVD) remains as the leading cause of mortality in the world despite the progressive decrease in last decades and this has been largely due to risk factors control and improvements of acute phases management[1-4]. This positive results has lead to a large increase in the prevalence of patients with chronic CVD in the population[5,6] that are high-risk patients and benefit from the highest risk factors control[2] as well as treatment implementation[6,7]. Moreover, the progressive ageing of the population and patients has contributed to increase the complexity of patients, with higher comorbidities and polivascular disease that have higher risk of future cardiovascular events[8,9].

Coronary heart disease (CHD) and heart failure (HF) represent more than half of the mortality attributed to CVD what highlights they relevancy in public health[1]. Several registries have depicted the changing profile of patients with CHD[4,10-12] or other types of CVD[3,8,13] but there scare evidence concerning the changing prevalence of CVD in outpatient clinics. With the objective of describing current profile of patients with CVD and assessing changes through last decade we compared two clinical registries performed in Spain in 1999 and 2009.

## MATERIALS AND METHODS

### Study design

The CARDIOTENS 2009 registry is a cross-sectional, multicentre and nationwide study of patients with risk factors or cardiovascular disease[14], with similar methodology of CARDIOTENS registry performed in 1999[15]; both studies were promoted the Working Group of Hypertension of the Spanish Society of Cardiology and endorsed by the Agencia de Investigación de la Soceidad Española de Cardiología (Research Agency of the Spanish Society of Cardiology). From the 32051 subjects include in the 1999 registry 6194 (19.3%) had established CVD and were compared to the 4639 (18.2% of the 25856) patients with established CVD collected in 2009.

As defined in the 1999 registry established CVD was defined as: (1) CHD if antecedents of angina, myocardial infarction, coronary revascularization or positive stress test were present; (2) HF if patients had at least one hospitalization with such as main diagnosis at discharge medical report as well as those with typical signs and symptoms of HF that had a compatible imagine diagnosis (X-ray or echocardiogram); and (3) atrial fibrillation (AF) if the diagnosis was present in a medical report or any electrocardiographic registry. A brief analysis of guideline-recommended treatments was performed based on the use of beta-blockers in patients with CHD, angiotensin converter-enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) in patients with HF, and oral anticoagulants in AF patients.

Investigators were randomly selected from primary care (88%) and cardiology outpatient clinics (12%). During the first week of November 2009 investigators included all consecutive patients that they attended; if patients had no risk factors or cardiovascular disease, only age. In patients with any risk factor or established cardiovascular disease a more extended protocol was performed in which all risk factors, clinical antecedents, treatments, physical examination and Inclusion criteria were: age $\geqslant$ 18 years, capability to access to full medical antecedents related to cardiovascular risk factors or events and allowance to participate by singing the informed consent. Exclusion criteria were addiction or consumption of illegal substances (cocaine, cannabis or other) or denial of the informed the consent. The study protocol and informed consent was approved by the ethics committee of the Hospital Universitario de San Juan (Alicante, Spain) and the Agencia of Investigación of the Spanish Society of Cardiology.

A specific collection data report in paper was designed for the study; a modification had to be added once printed and approved by the ethic committee due to an error in the codification of diuretics treatment. Electrocardiogram and biochemical determinations had to be obtained in within the last 6 mo. Blood pressure was measured after ten minutes of resting in the inclusion visit and two determinations were collected. Heart rate had to be measured during the physical examination.

### Variables definition

Hypertension was defined according to the 2007 European Society of Hypertension-European Society of Cardiology guidelines if 2 determinations of blood pressure were $\geqslant$ 140/90 mmHg or specific treatments with previous diagnosis were present[16]. Dyslipidemia was collected if any antecedent of such diagnosis or values of total cholesterol > 220 mg/dL or low-density lipoproteins > 160 mg/dL had been registered previously. The diagnosis of diabetes mellitus was accepted if it had been previously diagnosis in a medical report, specific drug-treatment or 2 consecutive glucose determinations were > 126 mg/dL. Obesity was considered for those with body mass index > 30 kg/m$^2$ and abdominal obesity if waist circumference was > 102 cm in men or > 88 cm in women. Chronic obstructive pulmonary disease was registered

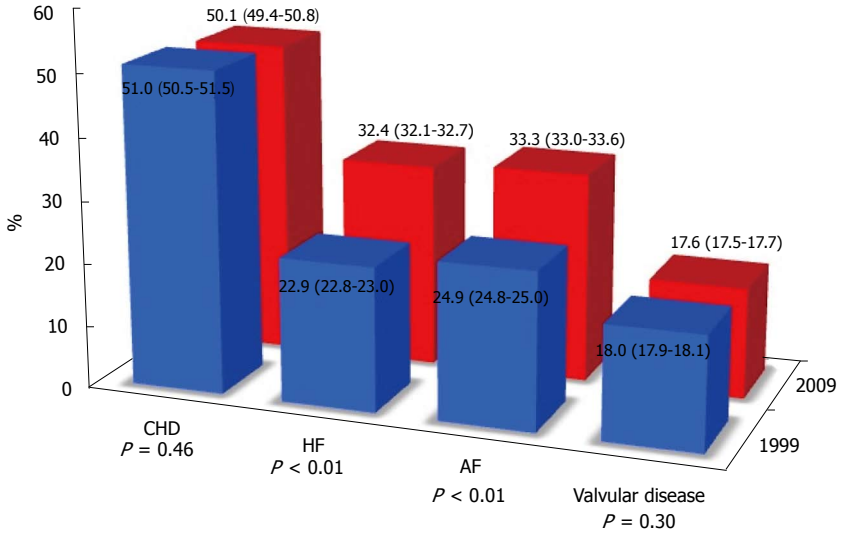



**Figure 1 Trends in cardiovascular disease prevalence between 1999 and 2009 in both CARDIOTENS registries.** AF: Atrial fibrillation; CHD: Coronary heart disease; HF: Heart failure. Data presented as percentage (99%CI).

if specific treatments were present or previous diagnosis was present. Glomerular filtration rate was assessed by the Modification of Diet in Renal Disease equation: (186 × creatinine$^{-1.154}$ × age$^{-0.203}$) (× 0.742 in women).

### Statistical analysis

Data management was made with statistical package SPSS 15.0 (SPSS Inc, Chicago, IL, United States) and 10.0/SE (Stata Corp, College Station, Tex). All variables had normal distribution so are presented as mean (standard deviation), except triglycerides that are presented as median (intercuartilic range); mean comparisons were made with ANOVA test and non-parametric $\chi^2$. Percentages were compared by *t*-Student and non-parametric Kolmogorov-Smirnov test were used for comparison of means and medians respectively. Comparisons of percentages between the 2 registries were performed by *t*-Student contrast using the analysis of variance of the estimated percentages of each registry. Statistical significance was accepted for $P < 0.05$.

## RESULTS

As presented in Table 1, patients of the 2009 registry had higher mean age, as well as higher prevalence of all risk factors except for current-smoking; the most significant raise was noted in patients aged > 70 or > 80 years. A significant reduction in mean systolic and diastolic BP was observed that lead to a 54.4% relative increase in blood pressure control. CHD was the most prevalent from of established CVD and its prevalence remained similar in both registries, as well as valvular heart disease that were the least prevalent CVD; a significant increase in the prevalence of HF (41.5% relative increase) and AF (33.7% relative increase) was observed (Figure 1).

We also observed a significant reduction in serum lipid levels although the prevalence of dyslipidemia increased between both registries (Table 2); in concordance, a large relative increase in the use of statins, as well as all cardiovascular treatments was observed between both registries, except nitrates (Table 3). Moreover, the use of guideline-recommended treatments, such as ACEI or ARB in patients with HF, beta-blockers in the setting of CHD or oral anticoagulants in patients with AF, increased 83.0%, 80.3% and 156.0% (Figure 2).

## DISCUSSION

The comparison of two large clinical registries performed with similar design and methodology allowed the description of risk factors and CVD prevalence evolution through last decade in Spain. Hypertension was the most prevalent risk factor in 1999 and was present in almost all patients with CVD 10 year later. Moreover, a relevant relative improvement in blood pressure control and guidelines-recommended treatments was observed, despite a slight reduction in mean systolic and diastolic blood pressure values. The prevalence of risk factors, mean age and medical treatments of patients included in 2009 was similar to contemporary registries[17-22] what reflects that this population might be representative of clinical relative.

Our results highlight that hypertension is present almost all patients with established CVD and is prevalence experienced a relative increase of 32% through last decade. This overwhelming rise can be explained by several facts; first, blood pressure is usually reported to be one the risk factors with poorest control what leads to a high prevalence of hypertension in patients with incident CVD[19,23]; second, the antecedent of hypertension does not impair the prognosis in the acute setting of CVD what leads to a increasing percentage of patients with hypertension and established CVD[24,25]; third, subjects with hypertension have benefit from a significantly higher decrease in cardiovascular mortality through last decades[21]; and fourth, population has experienced a relevant

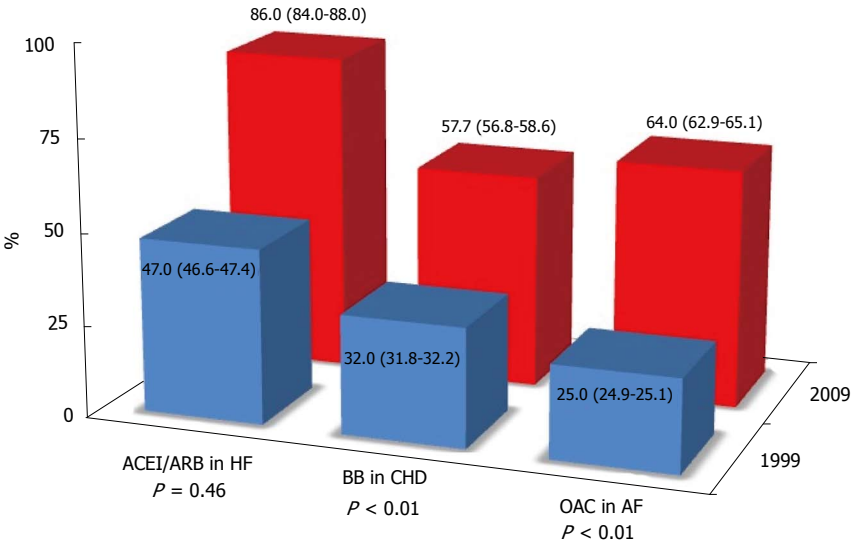



**Figure 2 Changes in use of guideline-recommended treatments in each compelling indication between 1999 and 2009 in both CARDIOTENS registries.** ACEI: Angiotensin-converter enzyme inhibitors; AF: Atrial fibrillation; ARB: Angiotensin-receptor blockers; CHD: Coronary heart disease; HF: Heart failure; OAC: Oral anticoagulation. Data presented as percentage (99%CI).

**Table 1 Comparative characteristics of the patients included in each registry**

| | 1999 | 2009 | *P* vaule |
|---|---|---|---|
| Number | 6194 | 4639 | |
| Age (yr) | 66.7 ± 10.8 | 70.6 ± 11.3 | < 0.01 |
| Age >70 yr | 45.1% (44.7-45.5) | 56.9% (56.0-57.8) | < 0.01 |
| Age > 80 yr | 13.0% (12.9-13.1) | 18.6% (18.5-18.7) | < 0.01 |
| Males | 54% (53.4-54.6) | 57.2% (56.3-58.1) | < 0.01 |
| BMI > 30 mg/m$^2$ | 30% (29.8-30.2) | 31.9% (31.6-32.2) | 0.02 |
| Hypertension | 65% (64.1-65.9) | 85.8% (83.8-87.8) | < 0.01 |
| Diabetes | 21% (20.9-21.1) | 38.2% (37.8-38.6) | < 0.01 |
| Dyslipidemia | 40% (39.7-40.3) | 59.2% (58.2-60.2) | < 0.01 |
| Current smokers | 20% (19.9-20.1) | 12.1% (12.0-12.2) | < 0.01 |
| Left ventricle hypertrophy | 24.0% (23.9-24.1) | 30.9% (30.6-31.2) | < 0.01 |
| Systolic BP (mmHg) | 138.2 ± 15.2 | 136.6 ± 16.0 | < 0.01 |
| Diastolic BP (mmHg) | 79.1 ± 10.9 | 78.6 ± 11.5 | < 0.01 |
| BP < 140/90 mmHg | 36.0% (35.7-36.3) | 55.6% (54.8-56.4) | < 0.01 |

Data collected from CARDIOTENS registries, Spain. Data are presented as mean ± SD or percentage (99%CI). BMI: Body mass index; BP: Blood pressure.

**Table 2 Lipid profile of patients included in each registry**

| | 1999 | 2009 | *P* vaule |
|---|---|---|---|
| Total cholesterol (mg/dL) | 218.3 | 196.3 (45.8) | < 0.01 |
| HDL (mg/dL) | 49.5 | 50.2 (15.3) | < 0.01 |
| LDL (mg/dL) | 145.1 | 117.0 (37.5) | < 0.01 |
| Triglycerides (mg/dL) | 142.6 | 145.1 (70.5) | 0.02 |

Data collected from CARDIOTENS registries, Spain. HDL: High-density lipoproteins; LDL: low-density lipoproteins.

**Table 3 Medical treatments in each registry**

| | 1999 | 2009 | *P* vaule |
|---|---|---|---|
| Antiplatelets | 42.0% (41.6-42.4) | 76.8% (75.2-78.4) | < 0.01 |
| Diuretics | 32.0% (31.8-32.2) | 4.4% (4.39-4.41) | < 0.01 |
| ACEI | 30.2% (30.0-30.4) | 43.6% (43.1-44.1) | < 0.01 |
| Statins | 27.0% (26.9-27.1) | 65.1% (63.9-66.3) | < 0.01 |
| CCB | 27.8% (27.6-28.0) | 36.7% (36.3-37.1) | < 0.01 |
| Nitrates | 25.0% (24.9-25.1) | 26.3% (26.1-26.5) | 0.06 |
| Beta-blockers | 22.2% (22.1-22.3) | 44.6% (44.1-45.1) | < 0.01 |
| Oral anticoagulants | 17.5% (17.4-17.6) | 27.0% (26.8-27.2) | < 0.01 |
| ARB | 11.1% (11.0-11.1) | 38.6% (38.2-39.0) | < 0.01 |
| Insulin | 5.1% (5.09-5.10) | 13.7% (13.6-13.8) | < 0.01 |
| Oral antidiabetics | 10.0% (9.9-10.1) | 43.7% (43.2-44.2) | < 0.01 |

Data collected from CARDIOTENS registries, Spain. Data presented as percentages (99%CI). ACEI: Angiotensin converter-enzyme inhibitors; ARB: Angiotensin receptor blockers; CCB: Calcium channel blockers.

increase in clinical features in the population that are closely related to high-blood pressure, as advanced age[16], obesity[26] or diabetes[27] predispose to a increasing pattern in hypertension prevalence. Our registry included stable patients with CVD and strongly supports these four key-points and provides not only a reasonable profile of patients with hypertension and CVD but, also, a clinical perspective of its evolution through last decade.

CHD remained as the most prevalent forms of established CVD but experienced no relative change through last decade; nevertheless, the presence of HF and AF increased largely; the study protocol on the 1999 registry did not other forms of CVD, such as stroke or peripheral arterial disease, and we could not obtain data of these two relevant clinical entities[1]. HF and AF have been clearly related to age and hypertension and have been reported to rise in the overall population steeply through last decades[1,28]. Blood pressure control has been outlined as major target for prevention of HF[29] and AF[30] especially by the use of ACEI or ARB[31] and target organ damage prevention. In contrast, mean age increased in the comparison between both registries and the prevalence of octogenarians reached almost 20%. The evidence of BP control and the optimal objective in elderly patients has been less studied[16]; only the Hypertension in the Very Elderly Trial[32] study was specifically designed to assess the benefit of BP control and target BP was < 150/80 mmHg. The study demonstrated both

significant improvement in cardiovascular events and mortality in patients actively treated with a diuretic and an ACEI. These results added to net benefit of ACEI or ARB in patients with established CVD might explain the large increase in the use of these treatments observed in our results.

Our results show at least two positive messages: the increase in blood pressure control and the improvement in medical treatments. All guideline-recommended treatments experienced significant increases, being the use of statins, ACEI and ARB the most prominent. The use of statins has spread to majority of patients with established CVD, especially in the patients with CHD[17,18,23] and its use in patients with HF is much lower because its clinical benefit in absence of underlying CHD is not clear. Nonetheless, our results highlighted a very relevant increase in the use of ACEI or ARB in patients with HF that agrees with previous reports of other registries[22,28]. The use of beta-blockers in patients with CHD also increased but only reached 57%, a very similar percentage of registries that included chronic and stable patients[6,17,18]. The increase in the oral anticoagulants in patients with AF was remarkable and treatment rate in our registry was similar to the last report of the European Heart Survey[33].

Our study has several limitations that deserve consideration, mainly derived from its design; for being a cross-sectional study it can only describe clinical associations and causal effects. Moreover, it only included consecutive patients that were attended in outpatient clinics and, therefore, our results are not representative of overall population. The study protocol did not include stroke or peripheral disease as established CVD and, therefore, we can not provide actualized data on these two relevant diseases. Finally, there was an error in the typing of diuretics and, therefore, data collection concerning this medication was not accurate.

In conclusion, the prevalence of all cardiovascular risk factors has increased in patients with established CVD, except smoking. Hypertension is most prevalent risk factors in these patients and a significant improvement in BP control has been achieved although it is still far from optimal goals. A relevant improvement in guideline-recommended treatments could be demonstrated, as well as major cardiovascular treatments, being the use of statins the most remarkable. We describe a positive trend in blood pressure control and guidelines-recommended treatments but there are still opportunities for further improvement.

## COMMENTS

### Background

Reduction in acute phase of cardiovascular disease (CVD) has lead to a progressive increase in patients with chronic CVD that are considered high-risk patients and mostly attended in outpatient clinics.

### Research frontiers

Risk factors control and medical treatment has been usually reported as lower in high-risk patients. Many registries have reported relevant increases in blood pressure or cholesterol control, although the changing pattern in clinical profile and risk factors control of patients with established cardiovascular disease has been far less studied.

### Innovations and breakthroughs

The prevalence of heart failure and atrial fibrillation has increased significantly through the last decade, meanwhile coronary heart disease remained as the most prevalent. Similarly, authors have noted an increase in mean age, especially in the percentage of elderly patients, and all risk factors but smoking. Risk factors control has increases as well as guidelines-recommended medical treatments.

### Applications

Out patients clinics should be prepared, focused and organized to attended more patients with heart failure or atrial fibrillation that have very specific considerations, such as weight-gain, symptoms control, medication use, anticoagulants complications.

### Terminology

Coronary heart disease: patients with the antecedent of myocardial infarction, angina, acute coronary syndromes or any kind of coronary revascularization.

### Peer review

This is a good descriptive study in which authors analyze changes in clinical profile and medical treatments of patients with cardiovascular disease. The results are interesting and highlight the increasing trend in the prevalence of heart failure and atrial fibrillation, two clinical entities that deserve very specific considerations in out-patient clinics.

## REFERENCES


1 **Go AS**, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Executive summary: heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation* 2013; **127**: 143-152 [PMID: 23283859 DOI: 10.1161/CIR.0b013e31828124ad]

2 **Perk J**, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren WM, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germanò G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvanne M, Scholte Op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F. [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)]. *G Ital Cardiol* (Rome) 2013; **14**: 328-392 [PMID: 23612326]

3 **Perk J**, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvänne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F. 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). *Eur Heart J* 2012; **33**: 1635-1701 [PMID: 22555213 DOI: 10.1093/eurheartj/ehs092]

4 **Baker DW**, Einstadter D, Thomas C, Cebul RD. Mortality trends for 23,505 Medicare patients hospitalized with heart failure in Northeast Ohio, 1991 to 1997. *Am Heart J* 2003; **146**: 258-264 [PMID: 12891193 DOI: 10.1016/S0002-8703(02)94784-8]

5 **Arós F**, Heras M, Vila J, Sanz H, Ferreira-González I, Perma-

nyer-Miralda G, Cuñat J, López-Bescós L, Cabadés A, Loma-Osorio A, Marrugat J. [Reduction in 28 days and 6 months of acute myocardial infarction mortality from 1995 to 2005. Data from PRIAMHO I, II and MASCARA registries]. *Rev Esp Cardiol* 2011; **64**: 972-980 [PMID: 21803474]

6 **Davies AR**, Smeeth L, Grundy EM. Contribution of changes in incidence and mortality to trends in the prevalence of coronary heart disease in the UK: 1996 2005. *Eur Heart J* 2007; **28**: 2142-2147 [PMID: 17636307 DOI: 10.1093/eurheartj/ehm272]

7 **Bhatt DL**, Eagle KA, Ohman EM, Hirsch AT, Goto S, Mahoney EM, Wilson PW, Alberts MJ, D'Agostino R, Liau CS, Mas JL, Röther J, Smith SC, Salette G, Contant CF, Massaro JM, Steg PG. Comparative determinants of 4-year cardiovascular event rates in stable outpatients at risk of or with atherothrombosis. *JAMA* 2010; **304**: 1350-1357 [PMID: 20805624 DOI: 10.1001/jama.2010.1322]

8 **Maron DJ**, Boden WE, O'Rourke RA, Hartigan PM, Calfas KJ, Mancini GB, Spertus JA, Dada M, Kostuk WJ, Knudtson M, Harris CL, Sedlis SP, Zoble RG, Title LM, Gosselin G, Nawaz S, Gau GT, Blaustein AS, Bates ER, Shaw LJ, Berman DS, Chaitman BR, Weintraub WS, Teo KK. Intensive multifactorial intervention for stable coronary artery disease: optimal medical therapy in the COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation) trial. *J Am Coll Cardiol* 2010; **55**: 1348-1358 [PMID: 20338496 DOI: 10.1016/j.jacc.2009.10.062]

9 **Fang J**, Mensah GA, Croft JB, Keenan NL. Heart failure-related hospitalization in the U.S., 1979 to 2004. *J Am Coll Cardiol* 2008; **52**: 428-434 [PMID: 18672162 DOI: 10.1016/j.jacc.2008.03.061]

10 **Cordero A**, Morillas P, Bertomeu-González V, Quiles J, Soria F, Guindo J, Mazón P, Anguita M, Rodríguez-Padial L, González-Juanatey JR, Bertomeu-Martínez V. Pathological ankle-brachial index is equivalent of advanced age in acute coronary syndromes. *Eur J Clin Invest* 2011; **41**: 1268-1274 [PMID: 21517830 DOI: 10.1111/j.1365-2362.2011.02533.x]

11 **Schiele F**, Hochadel M, Tubaro M, Meneveau N, Wojakowski W, Gierlotka M, Polonski L, Bassand JP, Fox KA, Gitt AK. Reperfusion strategy in Europe: temporal trends in performance measures for reperfusion therapy in ST-elevation myocardial infarction. *Eur Heart J* 2010; **31**: 2614-2624 [PMID: 20805111 DOI: 10.1093/eurheartj/ehq305]

12 **Fagring AJ**, Lappas G, Kjellgren KI, Welin C, Manhem K, Rosengren A. Twenty-year trends in incidence and 1-year mortality in Swedish patients hospitalised with non-AMI chest pain. Data from 1987-2006 from the Swedish hospital and death registries. *Heart* 2010; **96**: 1043-1049 [PMID: 20483906 DOI: 10.1136/hrt.2010.193748]

13 **Roger VL**, Weston SA, Gerber Y, Killian JM, Dunlay SM, Jaffe AS, Bell MR, Kors J, Yawn BP, Jacobsen SJ. Trends in incidence, severity, and outcome of hospitalized myocardial infarction. *Circulation* 2010; **121**: 863-869 [PMID: 20142444 DOI: 10.1161/CIRCULATIONAHA.109.897249]

14 **Otero-Raviña F**, Grigorian-Shamagian L, Fransi-Galiana L, Názara-Otero C, Fernández-Villaverde JM, del Alamo-Alonso A, Nieto-Pol E, de Santiago-Boullón M, López-Rodríguez I, Cardona-Vidal JM, Varela-Román A, González-Juanatey JR. Morbidity and mortality among heart failure patients in Galicia, N.W. Spain: the GALICAP Study. *Int J Cardiol* 2009; **136**: 56-63 [PMID: 18617282 DOI: 10.1016/j.ijcard.2008.04.025]

15 **Cordero A**, Bertomeu-Martínez V, Mazón P, Fácila L, Bertomeu-González V, Cosín J, Galve E, Núñez J, Lekuona I, González-Juanatey JR. [Factors associated with uncontrolled hypertension in patients with and without cardiovascular disease]. *Rev Esp Cardiol* 2011; **64**: 587-593 [PMID: 21640460 DOI: 10.1016/j.recesp.2011.03.008]

16 **Juanatey JR**, Ezquerra EA, Vidal JV, Caro JL, Acuña JG, Maqueda IG. [Impact of hypertension in cardiac diseases in Spain. The CARDIOTENS Study 1999]. *Rev Esp Cardiol* 2001; **54**: 139-149 [PMID: 11181302 DOI: 10.1157/030089320540002 20010139]

17 **Mancia G**, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Struijker Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Kjeldsen SE, Erdine S, Narkiewicz K, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Cifkova R, Dominiczak A, Fagard R, Heagerty AM, Laurent S, Lindholm LH, Mancia G, Manolis A, Nilsson PM, Redon J, Schmieder RE, Struijker-Boudier HA, Viigimaa M, Filippatos G, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Kiowski W, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Viigimaa M, Waeber B, Williams B, Zamorano JL. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2007; **28**: 1462-1536 [PMID: 17562668]

18 **Kotseva K**, Wood D, De Backer G, De Bacquer D, Pyörälä K, Keil U. Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries. *Lancet* 2009; **373**: 929-940 [PMID: 19286092 DOI: 10.1016/S0140-6736(09)60330-5]

19 **Cordero A**, Bertomeu-Martínez V, Mazón P, Quiles J, Aznar J, Bueno H. Differences in medical treatment of chronic coronary heart disease patients according to medical specialities. *Cardiovasc Ther* 2009; **27**: 173-180 [PMID: 19689616 DOI: 10.1111/j.1755-5922.2009.00093.x]

20 **Egan BM**, Zhao Y, Axon RN. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. *JAMA* 2010; **303**: 2043-2050 [PMID: 20501926 DOI: 10.1001/jama.2010.650]

21 **Falaschetti E**, Chaudhury M, Mindell J, Poulter N. Continued improvement in hypertension management in England: results from the Health Survey for England 2006. *Hypertension* 2009; **53**: 480-486 [PMID: 19204180 DOI: 10.1161/HYPERTENSIONAHA.108.125617]

22 **Ford ES**. Trends in mortality from all causes and cardiovascular disease among hypertensive and nonhypertensive adults in the United States. *Circulation* 2011; **123**: 1737-1744 [PMID: 21518989 DOI: 10.1161/CIRCULATIONAHA.110.005645]

23 **DiMartino LD**, Shea AM, Hernandez AF, Curtis LH. Use of guideline-recommended therapies for heart failure in the Medicare population. *Clin Cardiol* 2010; **33**: 400-405 [PMID: 20641116 DOI: 10.1002/clc.20760]

24 **Alberts MJ**, Bhatt DL, Mas JL, Ohman EM, Hirsch AT, Röther J, Salette G, Goto S, Smith SC, Liau CS, Wilson PW, Steg PG. Three-year follow-up and event rates in the international REduction of Atherothrombosis for Continued Health Registry. *Eur Heart J* 2009; **30**: 2318-2326 [PMID: 19720633 DOI: 10.1093/eurheartj/ehp355]

25 **Boersma E**, Pieper KS, Steyerberg EW, Wilcox RG, Chang WC, Lee KL, Akkerhuis KM, Harrington RA, Deckers JW, Armstrong PW, Lincoff AM, Califf RM, Topol EJ, Simoons ML. Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation. Results from an international trial of 9461 patients. The PURSUIT Investigators. *Circulation* 2000; **101**: 2557-2567 [PMID: 10840005 DOI: 10.1161/01.CIR.101.22.2557]

26 **Fácila L**, Bertomeu V, Núñez J, Sanchis J, Bodí V, Consuegra L, Bosch MJ, Roselló A, Martínez A, Blasco ML, Llàcer A. [Influence of antecedent of hypertension in patients with acute coronary syndrome without ST elevation]. *Med Clin*

*(Barc)* 2006; **126**: 121-124 [PMID: 16472494]
27 **Cordero A**, León M, Andrés E, Ordoñez B, Laclaustra M, Grima A, Pascual I, Luengo E, Civeira F, Pocoví M, Alegría E, Casasnovas JA. Gender differences in obesity related cardiovascular risk factors in Spain. *Prev Med* 2009; **48**: 134-139 [PMID: 19038283 DOI: 10.1016/j.ypmed.2008.10.024]
28 **Seshasai SR**, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; **364**: 829-841 [PMID: 21366474 DOI: 10.1056/NEJMoa1008862]
29 **Aranda JM**, Johnson JW, Conti JB. Current trends in heart failure readmission rates: analysis of Medicare data. *Clin Cardiol* 2009; **32**: 47-52 [PMID: 19143005 DOI: 10.1002/clc.20453]
30 **Schocken DD**, Benjamin EJ, Fonarow GC, Krumholz HM, Levy D, Mensah GA, Narula J, Shor ES, Young JB, Hong Y. Prevention of heart failure: a scientific statement from the American Heart Association Councils on Epidemiology and Prevention, Clinical Cardiology, Cardiovascular Nursing, and High Blood Pressure Research; Quality of Care and Outcomes Research Interdisciplinary Working Group; and Functional Genomics and Translational Biology Interdisciplinary Working Group. *Circulation* 2008; **117**: 2544-2565 [PMID: 18391114 DOI: 10.1161/CIRCULATIONAHA.107.188965]
31 **Lip GY**, Frison L, Grind M. Effect of hypertension on anticoagulated patients with atrial fibrillation. *Eur Heart J* 2007; **28**: 752-759 [PMID: 17289744 DOI: 10.1093/eurheartj/ehl504]
32 **Schneider MP**, Hua TA, Böhm M, Wachtell K, Kjeldsen SE, Schmieder RE. Prevention of atrial fibrillation by Renin-Angiotensin system inhibition a meta-analysis. *J Am Coll Cardiol* 2010; **55**: 2299-2307 [PMID: 20488299 DOI: 10.1016/j.jacc.2010.01.043]
33 **Beckett NS**, Peters R, Fletcher AE, Staessen JA, Liu L, Dumitrascu D, Stoyanovsky V, Antikainen RL, Nikitin Y, Anderson C, Belhani A, Forette F, Rajkumar C, Thijs L, Banya W, Bulpitt CJ. Treatment of hypertension in patients 80 years of age or older. *N Engl J Med* 2008; **358**: 1887-1898 [PMID: 18378519 DOI: 10.1056/NEJMoa0801369]
34 **Nieuwlaat R**, Capucci A, Lip GY, Olsson SB, Prins MH, Nieman FH, López-Sendón J, Vardas PE, Aliot E, Santini M, Crijns HJ. Antithrombotic treatment in real-life atrial fibrillation patients: a report from the Euro Heart Survey on Atrial Fibrillation. *Eur Heart J* 2006; **27**: 3018-3026 [PMID: 16731536 DOI: 10.1093/eurheartj/ehl015]

**P- Reviewers** Chawla M, Tan XR **S- Editor** Gou SX
**L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.295

*World J Cardiol* 2013 August 26; 5(8): 295-304
ISSN 1949-8462 (online)


BRIEF ARTICLE

# Central obesity in Yemeni children: A population based cross-sectional study

Mohamed Bamoshmoosh, Luciano Massetti, Hameed Aklan, Mahdi Al-Karewany, Husni Al Goshae, Pietro Amedeo Modesti

Mohamed Bamoshmoosh, Hameed Aklan, Mahdi Al-Karewany, Husni Al Goshae, University of Science and Technology, Sana'a 15201, Yemen
Luciano Massetti, Institute of Biometeorology, National Research Council, 50134 Florence, Italy
Pietro Amedeo Modesti, Department of Clinical and Experimental Medicine, University of Florence, Azienda Ospedaliero Universitaria Careggi, 50134 Florence, Italy
Author contributions: Bamoshmoosh M and Modesti PA drafted the manuscript; Bamoshmoosh M, Massetti L, Aklan H, Al-Karewany M, Al Goshae H and Modesti PA were involved in the conception and design of the study; Aklan H, Al-Karewany M and Al Goshae H coordinated data collection for the study; Modesti PA wrote the statistical analysis plan and provided statistical advice; Massetti L conducted the main analyses; Modesti PA is the guarantor.
Supported by HYpertension and Diabetes in Yemen Project is part of the Executive Programme of Scientific and Technological Cooperation between Italy and Yemen for the years 2006-2009; MIUR (Direzione Generale per le strategie e lo sviluppo dell' internazionalizzazione della ricerca scientifica e tecnologica), Rome, Italy; and Menarini International Operations Luxembourg SA
Correspondence to: Pietro Amedeo Modesti, MD, PhD, Department of Clinical and Experimental Medicine, University of Florence, Azienda Ospedaliero Universitaria Careggi, Largo Brambilla 3, 50134 Florence, Italy. pamodesti@unifi.it
Telephone: +55-7-949376-432758 Fax: +55-7-949376
Received: March 6, 2013 Revised: June 20, 2013
Accepted: July 25, 2013
Published online: August 26, 2013

## Abstract

**AIM:** To establish percentile curves explore prevalence and correlates of central obesity among Yemeni children in a population based cross-sectional study.

**METHODS:** A representative sample of 3114 Yemeni children (1564 boys, 1550 girls) aged 6-19 years participating in the HYpertension and Diabetes in Yemen study was studied. Data collection was conducted at home by survey teams composed of two investigators of both genders. Study questionnaire included questions about demographics, lifestyle, and medical history. Anthropometric measurements included body weight, height, waist circumference (WC) and hip circumferences. Waist to hip ratio (WHR) and waist-to-height ratio (WHtR) were then calculated. Age- and gender-specific percentiles (reference curves) of WC, WHR and WHtR were obtained with the laser mass spectroscopy method. The independent predictors of central obesity defined as (1) WC percentile $\geqslant 90^{th}$; (2) WHtR $\geqslant 0.5$; or (3) WC percentile $\geqslant 90^{th}$ and WHtR $\geqslant 0.5$, were identified at multivariate logistic regression analysis adjusted for age, gender, urban/rural location, years of school education, sedentary/active life-style.

**RESULTS:** Percentile curves for WC, WHR and WHtR are presented. Average WC increased with age for both genders. Boys had a higher WC than girls until early adolescence and thereafter girls had higher values than boys. WHR decreased both in boys and girls until early adolescence. Thereafter while in boys it plateaued in girls it continued to decrease. Mean WHtR decreased until early adolescence with no gender related differences and thereafter increased more in girls than in boys towards adult age. Prevalence of central obesity largely varied according to the definition used which was 10.9% for WC $\geqslant 90^{th}$ percentile, 18.3% for WHtR $\geqslant 0.5$, and 8.6% when fulfilling both criteria. At adjusted logistic regression WC $\geqslant 90^{th}$ percentiles and WHtR $\geqslant 0.5$ were less prevalent in rural than in urban areas (OR = 0.52, 95%CI: 0.41-0.67 and 0.66, 0.54-0.79 respectively), being more prevalent in children with sedentary lifestyle rather than an active one (1.52, 95%CI: 1.17-1.98 and 1.42, 95%CI: 1.14-1.75, respectively).

**CONCLUSION:** Yemeni children central obesity indices

percentile curves are presented. Central obesity prevalence varied according to the definition used and was more prevalent in urban sedentary subjects.



**Key words:** Central obesity; Waist circumference; Waist-to-height ratio; Waist to hip ratio; Developing countries

**Core tip:** This study presents the first central obesity percentile curves of waist circumference (WC), waist-to-height ratio (WHtR) and waist to hip ratio (WHR) for Yemeni children aged six to nineteen years. WC, WHtR and WHR changed similarly in girls and boys until early adolescence. Thereafter, differently from what observed in Western countries, changes increased more in girls than in boys. Prevalence of central obesity in Yemeni children is low, being associated with urbanization and sedentary lifestyle, and varied according to the definition used: (1) WC percentile $\geqslant 90^{th}$ (10.9%); (2) WHtR $\geqslant 0.5$ (18.3%); (3) WC percentile $\geqslant 90^{th}$ and WHtR $\geqslant 0.5$ (8.6%).

Bamoshmoosh M, Massetti L, Aklan H, Al-Karewany M, Al Goshae H, Modesti PA. Central obesity in Yemeni children: A population based cross-sectional study. *World J Cardiol* 2013; 5(8): 295-304 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/295.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.295

## INTRODUCTION

Childhood obesity is a matter of growing concern not only in developed but also in developing countries[1]. In the Middle Eastern Crescent (MEC) rates of adolescent overweight and obesity, assessed by the use of body mass index (BMI), are among the highest in the world[2]. The same world area is also characterized by a high prevalence of childhood central obesity as assessed by measuring waist circumference (WC)[3,4]. WC was consistently reported as a more sensitive indicator than BMI of metabolic abnormalities[5], and insulin resistance[6], also at young ages.

Measurements of BMI or WC in children have to be expressed in relation to their sex and age peers, and age and gender specific reference values are required for the diagnosis. Differently from WC, waist to hip ratio (WHR) and BMI a recently proposed index, waist-to-height ratio (WHtR), is only weakly associated with age, and gender[7,8]. Different studies are suggesting a single cut-off value for defining central obesity (WHtR $\geqslant 0.5$)[9]. However, ethnicity and environmental differences might influence body proportions, and it was suggested to use national references to control for variations between populations[10].

Considerable variation in the prevalence of risk factors was reported among different MEC countries[11] and few information is available in the pediatric population. According to a single study performed in Lahore (Pakistan), 16% of children aged 5-12 years had WHtR above the cut off value of 0.5[12]. HYpertension and Diabetes in Yemen (HYDY) study was thus also designed to provide age- and gender-specific WC, WHR and WHtR smoothed percentiles, and to explore prevalence and correlates of central obesity, among Yemeni children and adolescents aged six to nineteen years.

## MATERIALS AND METHODS

### *Study sites and study population*

Target population of the study was the population of the country aged 6-19 years. A representative sample of 3114 Yemeni children (1564 boys, 1550 girls) aged 6 to 19 years (median age 13.5 years) participating in the HYDY survey was studied[13]. The survey used a multi-stage stratified sampling method to select households as the setting for data collection[13]. Briefly in the first stage, Yemen was stratified into three areas, the capital area, the inland, and the coastal area. The governorate of Sana'a (capital area), the governorate of Taizz (inland), and the governorates of Al Hudaydah and Hadramaut (coast) were selected to be representative of the geographic, economic, and climatic characteristics of the three areas. The number of subjects in each area was estimated using the preliminary data provided by the United Nations Population Fund that is the same source used to stratify Yemen population by age and gender[14]. In the second stage, rural and city regions were identified from each study area. In the third stage, districts were arbitrarily identified within each urban and rural region, boundaries being defined using local maps or in consultation with the local health workers. Households were selected because in developing countries not all children in the age groups of interest may have access to school. The survey was completed within 16 mo. The response rate for subjects aged 6-19 years was 96% in urban and 97% in rural locations. The study was approved by the Ethical Committee of the University of Science and Technology, Sana'a, Yemen. Informed consent was obtained from participants and their parents before data collection. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2004.

### *Data collection*

Data collection was conducted at home by centrally trained survey teams composed of two investigators of both genders. Children were evaluated between February 2008 and May 2009. Study questionnaire included questions about demographics, lifestyle, and medical history. Anthropometric measurements were taken on standing participants wearing light clothing and without shoes using standard techniques[13]. In the HYDY survey we used a pre-calibrated digital Laica PS6010 scale with a 150 kg capacity (accuracy 100 g) which was frequently checked

**Table 1 Descriptive statistics by age group of the 3114 study participants are reported**

| Age (yr) | *n* | Weight | Height (cm) | BMI (kg/m$^2$) | WC | WHtR | HC | WHR |
|---|---|---|---|---|---|---|---|---|
| Boys | | | | | | | | |
| 6 | 47 | 17.4 ± 3.5 | 104.7 ± 8.4 | 16.0 ± 3.6 | 48.6 ± 6.5 | 0.47 ± 0.06 | 55.3 ± 9.1 | 0.88 ± 0.08 |
| 7 | 90 | 18.8 ± 3.5 | 109.9 ± 8.8 | 15.7 ± 2.8 | 50.8 ± 5.7 | 0.47 ± 0.07 | 57.2 ± 6.6 | 0.89 ± 0.07 |
| 8 | 99 | 21.3 ± 4.5 | 114.7 ± 9.4 | 16.4 ± 3.8 | 52.2 ± 7.1 | 0.46 ± 0.07 | 59.6 ± 7.6 | 0.88 ± 0.08 |
| 9 | 101 | 24.3 ± 7.4 | 120.5 ± 10.6 | 16.8 ± 4.6 | 55.6 ± 8.5 | 0.46 ± 0.08 | 63.8 ± 8.9 | 0.87 ± 0.07 |
| 10 | 132 | 26.8 ± 6.6 | 126.9 ± 10 | 16.9 ± 4.1 | 56.3 ± 7.5 | 0.45 ± 0.06 | 64.6 ± 9.1 | 0.88 ± 0.09 |
| 11 | 102 | 26.7 ± 4.8 | 129.3 ± 7.9 | 16.0 ± 2.8 | 57.0 ± 7.0 | 0.44 ± 0.05 | 65.2 ± 7.5 | 0.88 ± 0.07 |
| 12 | 157 | 29.9 ± 7.1 | 133.6 ± 8.4 | 16.7 ± 3.5 | 57.1 ± 8.4 | 0.43 ± 0.06 | 67.3 ± 9.0 | 0.85 ± 0.07 |
| 13 | 116 | 34.7 ± 8.2 | 141.5 ± 9.9 | 17.2 ± 3.4 | 61.4 ± 8.2 | 0.43 ± 0.05 | 71.7 ± 9.1 | 0.86 ± 0.07 |
| 14 | 158 | 39.2 ± 8.5 | 148.1 ± 9.7 | 17.8 ± 2.9 | 62.6 ± 8.1 | 0.42 ± 0.05 | 73.3 ± 8.5 | 0.86 ± 0.07 |
| 15 | 112 | 43.2 ± 11.7 | 150.3 ± 12.8 | 18.9 ± 4.0 | 66.8 ± 9.5 | 0.45 ± 0.06 | 77.2 ± 10.2 | 0.87 ± 0.08 |
| 16 | 100 | 47.3 ± 8.9 | 155.2 ± 9.6 | 19.7 ± 3.9 | 67.9 ± 9.2 | 0.44 ± 0.06 | 79.9 ± 9.3 | 0.85 ± 0.10 |
| 17 | 119 | 50.1 ± 9.3 | 160.1 ± 10.6 | 19.7 ± 3.9 | 68.1 ± 9.4 | 0.43 ± 0.06 | 80.3 ± 9.7 | 0.85 ± 0.08 |
| 18 | 133 | 51.4 ± 9.2 | 160.0 ± 11.1 | 20.1 ± 3.2 | 67.9 ± 8.7 | 0.43 ± 0.05 | 80.3 ± 9.1 | 0.85 ± 0.10 |
| 19 | 98 | 53.8 ± 11.3 | 161.3 ± 11.2 | 20.7 ± 4.0 | 70.3 ± 11.9 | 0.44 ± 0.07 | 82.8 ± 12.2 | 0.85 ± 0.08 |

Sample sizes, mean ± SD for weight, height, body mass index (BMI), waist circumference (WC), waist-to-height ratio (WHtR), hip circumference (HC), and waist to hip ratio (WHR) for Yemeni boys 6-19 years of age ($n$ = 3114).

by a known-weighing object. The CV for inter- and intra-observer effects for most anthropometric measures was < 5%. Height was measured to the nearest 0.5 cm using a stadiometer. WC was measured with a non-elastic tape positioned at a point midway between the lower border of the rib cage and the top of the iliac crest, and hip circumference (HC) was measured at the widest part of the hip at the level of the greater trochanter. WC and HC were measured to the nearest 0.5 cm and WHR and WHtR were then calculated.

BMI was computed as the weight in kilograms divided by the square of the height in meters. Central obesity was evaluated by analyzing WHtR ≥ 0.5, WC ≥ 90$^{th}$ percentile and the combination of both WHtR ≥ 0.5 and WC ≥ 90$^{th}$ percentile. Overweight and obesity were also defined as having a BMI above the age and sex-specific thresholds of the international obesity task force (IOTF) respectively the equivalent of BMI > 25 kg/m$^2$ and the equivalent of BMI > 30 kg/m$^2$[15].

### Statistical analysis

Description and validation of the database can be found elsewhere[16]. Data were preliminary checked for outliers using a cut-off of ± 5 SD of the corresponding age and sex Z-scores[17]. Smoothed age (by year) and gender-specific percentiles (3$^{rd}$, 5$^{th}$, 10$^{th}$, 25$^{th}$, 50$^{th}$, 75$^{th}$, 90$^{th}$, 95$^{th}$, and 97$^{th}$) for WC, WHR and WHtR were then constructed by means of the Box-Cox normal or laser mass spectroscopy (LMS) method of centiles estimation[18,19].

The LMS method summarizes the growth reference curve with three curves representing the median (M), the coefficient of variation (S) and the power to remove skewness from the data (L) by age and was implemented in the Generalized Additive Model for Location, Scale and Shape (GAMLSS) package included in R 2.14.0 software for Windows. In LMS method, GAMLSS parameters and the parameters of Box-Cox power exponential distribution were used for model fitting to data. These reference curves were fitted on the original data and the best fit was used to construct smoothed percentile curves. After the application of the BoxCox power transformation the data at each age were normally distributed and the points on each centile curve were defined in terms of the formula: $M = (1+LSz)^{1/L}$ where L, M, and S are values of the fitted curves at each age, and z indicates the z score for the required centile.

WC, WHR, and WHtR differences between genders were tested within age groups using Mann-Whitney *U*-test. Data are expressed as crude values. Comparisons were performed by using logistic regression with adjustment for confounding variables including age (years), gender, years of school education, urban/rural residency and sedentary/active lifestyle. Results are expressed as adjusted odd ratio (OR) with 95% confidence interval (CI). Test of hypothesis was done at significance level 0.05 two sided. SPSS software, version 19.0 (SPSS Inc., Chicago., IL, United States) was used for statistical comparisons.

## RESULTS

Descriptive statistics for weight, height, BMI, WC, WHtR, HC, and WHR by age group of the 3114 study participants are reported in Table 1. Age and gender specific WC, WHR and WHtR smoothed percentiles are presented in Tables 2-4 respectively. Mean BMI increased with age in both genders. However, girls aged 11 years or more had BMI values higher than boys (19.8 kg/m$^2$, 95%CI: 19.6-20.0 and 18.4 kg/m$^2$, 95%CI: 18.2-18.7 for girls and boys respectively). Overall 13.9% of participants (females 15.8%; 95%CI: 14.0-17.6; males 12.1%; 95%CI: 10.5 to 13.7) were overweight according to the IOTF criteria, 5.0% being obese (females 5.7%; 95%CI: 4.6-6.9; males 4.2%; 95%CI: 3.2-5.2).

WC increased with age among both boys and girls

**Table 2 Age, and gender specific smoothed waist circumference percentiles for Yemeni children and adolescents aged 6 to 19 years**

| Age (yr) | *n* | 3rd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97th |
|---|---|---|---|---|---|---|---|---|---|---|
| Boys | | | | | | | | | | |
| 6 | 47 | 36.60% | 38.50% | 41.30% | 45.40% | 48.90% | 52.50% | 57.20% | 60.70% | 63.30% |
| 7 | 90 | 37.60% | 39.70% | 42.60% | 46.80% | 50.40% | 54.10% | 58.90% | 62.50% | 65.20% |
| 8 | 99 | 38.80% | 40.90% | 43.90% | 48.20% | 52.00% | 55.80% | 60.80% | 64.50% | 67.30% |
| 9 | 101 | 40.00% | 42.20% | 45.30% | 49.80% | 53.60% | 57.60% | 62.70% | 66.50% | 69.40% |
| 10 | 132 | 41.20% | 43.40% | 46.60% | 51.20% | 55.10% | 59.20% | 64.40% | 68.40% | 71.30% |
| 11 | 102 | 42.20% | 44.50% | 47.70% | 52.50% | 56.50% | 60.70% | 66.10% | 70.10% | 73.10% |
| 12 | 157 | 43.40% | 45.80% | 49.10% | 54.00% | 58.10% | 62.40% | 68.00% | 72.10% | 75.20% |
| 13 | 116 | 44.90% | 47.40% | 50.80% | 55.80% | 60.10% | 64.60% | 70.30% | 74.70% | 77.90% |
| 14 | 158 | 46.70% | 49.20% | 52.80% | 58.00% | 62.40% | 67.10% | 73.00% | 77.50% | 80.80% |
| 15 | 112 | 48.40% | 51.00% | 54.70% | 60.10% | 64.70% | 69.50% | 75.70% | 80.30% | 83.80% |
| 16 | 100 | 49.70% | 52.40% | 56.30% | 61.80% | 66.60% | 71.50% | 77.80% | 82.60% | 86.20% |
| 17 | 119 | 50.80% | 53.50% | 57.40% | 63.10% | 67.90% | 73.00% | 79.40% | 84.30% | 87.90% |
| 18 | 133 | 51.60% | 54.30% | 58.30% | 64.10% | 69.00% | 74.10% | 80.70% | 85.60% | 89.30% |
| 19 | 98 | 52.30% | 55.10% | 59.10% | 65.00% | 70.00% | 75.20% | 81.80% | 86.80% | 90.60% |
| Girls | | | | | | | | | | |
| 6 | 51 | 34.30% | 36.50% | 39.70% | 44.40% | 48.70% | 53.10% | 58.00% | 61.30% | 63.60% |
| 7 | 79 | 35.10% | 37.40% | 40.60% | 45.50% | 49.90% | 54.30% | 59.30% | 62.70% | 65.10% |
| 8 | 86 | 36.00% | 38.30% | 41.70% | 46.60% | 51.10% | 55.70% | 60.80% | 64.30% | 66.80% |
| 9 | 121 | 37.10% | 39.40% | 42.90% | 48.00% | 52.60% | 57.30% | 62.60% | 66.20% | 68.70% |
| 10 | 164 | 38.40% | 40.80% | 44.40% | 49.60% | 54.50% | 59.30% | 64.70% | 68.50% | 71.10% |
| 11 | 123 | 39.80% | 42.40% | 46.10% | 51.50% | 56.50% | 61.60% | 67.20% | 71.10% | 73.80% |
| 12 | 124 | 41.40% | 44.00% | 47.90% | 53.50% | 58.70% | 640% | 69.80% | 73.90% | 76.70% |
| 13 | 132 | 43.00% | 45.70% | 49.70% | 55.60% | 61.00% | 66.50% | 72.60% | 76.70% | 79.70% |
| 14 | 130 | 44.70% | 47.50% | 51.70% | 57.80% | 63.40% | 69.10% | 75.40% | 79.80% | 82.80% |
| 15 | 102 | 46.20% | 49.20% | 53.50% | 59.80% | 65.60% | 71.50% | 78.00% | 82.50% | 85.70% |
| 16 | 108 | 47.40% | 50.50% | 54.90% | 61.40% | 67.30% | 73.40% | 80.10% | 84.70% | 87.90% |
| 17 | 97 | 48.40% | 51.50% | 55.90% | 62.60% | 68.70% | 74.80% | 81.60% | 86.30% | 89.60% |
| 18 | 128 | 49.10% | 52.30% | 56.90% | 63.60% | 69.80% | 76.00% | 83.00% | 87.70% | 91.10% |
| 19 | 105 | 49.80% | 53.00% | 57.60% | 64.50% | 70.70% | 77.10% | 84.10% | 88.90% | 92.30% |

Age, and gender specific smoothed waist circumference percentiles for Yemeni children and adolescents aged 6- 19 years ($n$ = 3114).

(Table 1). Boys had a non-significantly higher WC than girls until early adolescence (54.1 ± 7.7 cm *vs* 53.1 ± 8.7 cm, $P$ = 0.09 respectively for subjects aged ⩽ 11 years). Thereafter girls had higher WC values than boys (65.5 ± 10.7 cm *vs* 64.8 ± 10.0 cm, $P$ < 0.05 respectively for subjects aged > 11 years). Girls had lower 50th and 90th WC than boys in younger ages (6-11 years), but higher in older ages (12-19 years) (Table 2) (Figure 1A and D).

Mean WHR decreased with age among both boys and girls until early adolescence. Thereafter values plateaued in boys whereas in girls it continued to decrease. Boys had a higher WHR than girls in early adolescence (0.88 ± 0.08 *vs* 0.87 ± 0.09, $P$ < 0.01 respectively for subjects aged ⩽ 11 years) as well as thereafter (0.85 ± 0.08 *vs* 0.84 ± 0.09, $P$ < 0.01 respectively for subjects aged > 11 years). Girls always had lower 50th and 90th WHR than boys (Table 3) (Figure 1B and E).

Mean WHtR was slightly higher among boys than girls for subjects aged ⩽ 11 years (0.455 ± 0.067 and 0.448 ± 0.075 respectively, $P$ = 0.07). Thereafter WHtR values were higher in girls (0.450 ± 0.063) than in boys (0.431 ± 0.059, $P$ < 0.01). WHtR 50th percentile in both sexes decreased from the age of 6 years reaching the minimum at the age of 13 years, increasing thereafter mainly in girls. Girls had higher 50th and 90th WHtR percentiles than boys between the age of 14-19 years ($P$ < 0.05) (Table 4) (Figure 1C and F).

Prevalence of subjects with WC ⩾ 90th percentile, WHtR greater than 0.5, and of those fulfilling both criteria were 10.9%, 18.3%, and 8.6% in the overall population. More precisely 10.8% of girls (95%CI: 9.2-12.3), 11.0% of boys (95%CI: 9.4-12.5) had WC ⩾ 90th percentile; 21.7% of girls (95%CI: 19.6-23.7), 14.9% of boys (95%CI: 13.1-16.6) had WHtR ⩾ 0.5; 9.5% of girls (8.0-11.0), 7.8% of boys (95%CI: 6.4-9.1) had both WC ⩾ 90th percentile and WHtR ⩾ 0.5. Characteristics more frequently associated with reported criteria for central obesity were investigated at multivariate logistic regression, main results being reported in Table 5. In particular WC ⩾ 90th percentiles and WHtR ⩾ 0.5 were less prevalent in rural than in urban areas (OR = 0.52, 95%CI: 0.41-0.67 and 0.66, 0.54-0.79 respectively), being more prevalent in children with sedentary lifestyle (OR = 1.52, 95%CI: 1.17-1.98 and 1.42, 1.14-1.75 respectively). Differences by gender were evident only when considering a cut-off which is independent from the Yemeni population (WHtR ⩾ 0.5). A minor association was observed between years of school education and WC ⩾ 90th.

## DISCUSSION

This study, to our knowledge provides the first gender-

**Table 3 Age, and gender specific smoothed waist hip ratio percentiles for Yemeni children and adolescents aged 6 to 19 years**

| Age (yr) | *n* | 3th | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97th |
|---|---|---|---|---|---|---|---|---|---|---|
| Boys | | | | | | | | | | |
| 6 | 47 | 0.73 | 0.76 | 0.79 | 0.84 | 0.89 | 0.93 | 0.99 | 1.03 | 1.05 |
| 7 | 90 | 0.73 | 0.75 | 0.79 | 0.84 | 0.88 | 0.93 | 0.98 | 1.02 | 1.04 |
| 8 | 99 | 0.72 | 0.75 | 0.78 | 0.83 | 0.88 | 0.92 | 0.97 | 1.01 | 1.04 |
| 9 | 101 | 0.72 | 0.74 | 0.78 | 0.83 | 0.87 | 0.92 | 0.97 | 1.01 | 1.03 |
| 10 | 132 | 0.72 | 0.74 | 0.77 | 0.82 | 0.86 | 0.91 | 0.96 | 1.00 | 1.03 |
| 11 | 102 | 0.71 | 0.73 | 0.77 | 0.82 | 0.86 | 0.90 | 0.96 | 0.99 | 1.02 |
| 12 | 157 | 0.71 | 0.73 | 0.76 | 0.81 | 0.85 | 0.90 | 0.95 | 0.99 | 1.01 |
| 13 | 116 | 0.70 | 0.73 | 0.76 | 0.81 | 0.85 | 0.90 | 0.95 | 0.98 | 1.01 |
| 14 | 158 | 0.70 | 0.73 | 0.76 | 0.81 | 0.85 | 0.89 | 0.95 | 0.98 | 1.01 |
| 15 | 112 | 0.70 | 0.73 | 0.76 | 0.81 | 0.85 | 0.89 | 0.95 | 0.98 | 1.01 |
| 16 | 100 | 0.70 | 0.72 | 0.76 | 0.80 | 0.85 | 0.89 | 0.94 | 0.98 | 1.01 |
| 17 | 119 | 0.70 | 0.72 | 0.76 | 0.80 | 0.85 | 0.89 | 0.94 | 0.98 | 1.00 |
| 18 | 133 | 0.70 | 0.72 | 0.76 | 0.80 | 0.85 | 0.89 | 0.94 | 0.98 | 1.00 |
| 19 | 98 | 0.70 | 0.73 | 0.76 | 0.81 | 0.85 | 0.90 | 0.95 | 0.98 | 1.01 |
| Girls | | | | | | | | | | |
| 6 | 51 | 0.70 | 0.73 | 0.77 | 0.82 | 0.87 | 0.93 | 0.98 | 1.01 | 1.04 |
| 7 | 79 | 0.70 | 0.73 | 0.76 | 0.82 | 0.87 | 0.92 | 0.98 | 1.01 | 1.03 |
| 8 | 86 | 0.70 | 0.72 | 0.76 | 0.81 | 0.87 | 0.92 | 0.97 | 1.00 | 1.03 |
| 9 | 121 | 0.69 | 0.72 | 0.75 | 0.81 | 0.86 | 0.91 | 0.96 | 1.00 | 1.02 |
| 10 | 164 | 0.69 | 0.71 | 0.75 | 0.80 | 0.86 | 0.91 | 0.96 | 0.99 | 1.02 |
| 11 | 123 | 0.68 | 0.71 | 0.75 | 0.80 | 0.85 | 0.90 | 0.95 | 0.99 | 1.01 |
| 12 | 124 | 0.68 | 0.71 | 0.74 | 0.80 | 0.85 | 0.90 | 0.95 | 0.98 | 1.01 |
| 13 | 132 | 0.68 | 0.70 | 0.74 | 0.79 | 0.84 | 0.90 | 0.95 | 0.98 | 1.00 |
| 14 | 130 | 0.68 | 0.70 | 0.74 | 0.79 | 0.84 | 0.89 | 0.94 | 0.98 | 1.00 |
| 15 | 102 | 0.67 | 0.70 | 0.73 | 0.79 | 0.84 | 0.89 | 0.94 | 0.97 | 0.99 |
| 16 | 108 | 0.67 | 0.69 | 0.73 | 0.78 | 0.83 | 0.88 | 0.93 | 0.97 | 0.99 |
| 17 | 97 | 0.67 | 0.69 | 0.73 | 0.78 | 0.83 | 0.88 | 0.93 | 0.96 | 0.98 |
| 18 | 128 | 0.66 | 0.69 | 0.72 | 0.78 | 0.83 | 0.88 | 0.93 | 0.96 | 0.98 |
| 19 | 105 | 0.66 | 0.68 | 0.72 | 0.77 | 0.82 | 0.87 | 0.92 | 0.95 | 0.97 |

Age, and gender specific smoothed waist hip ratio percentiles for Yemeni children and adolescents aged 6-19 years ($n$ = 3114).

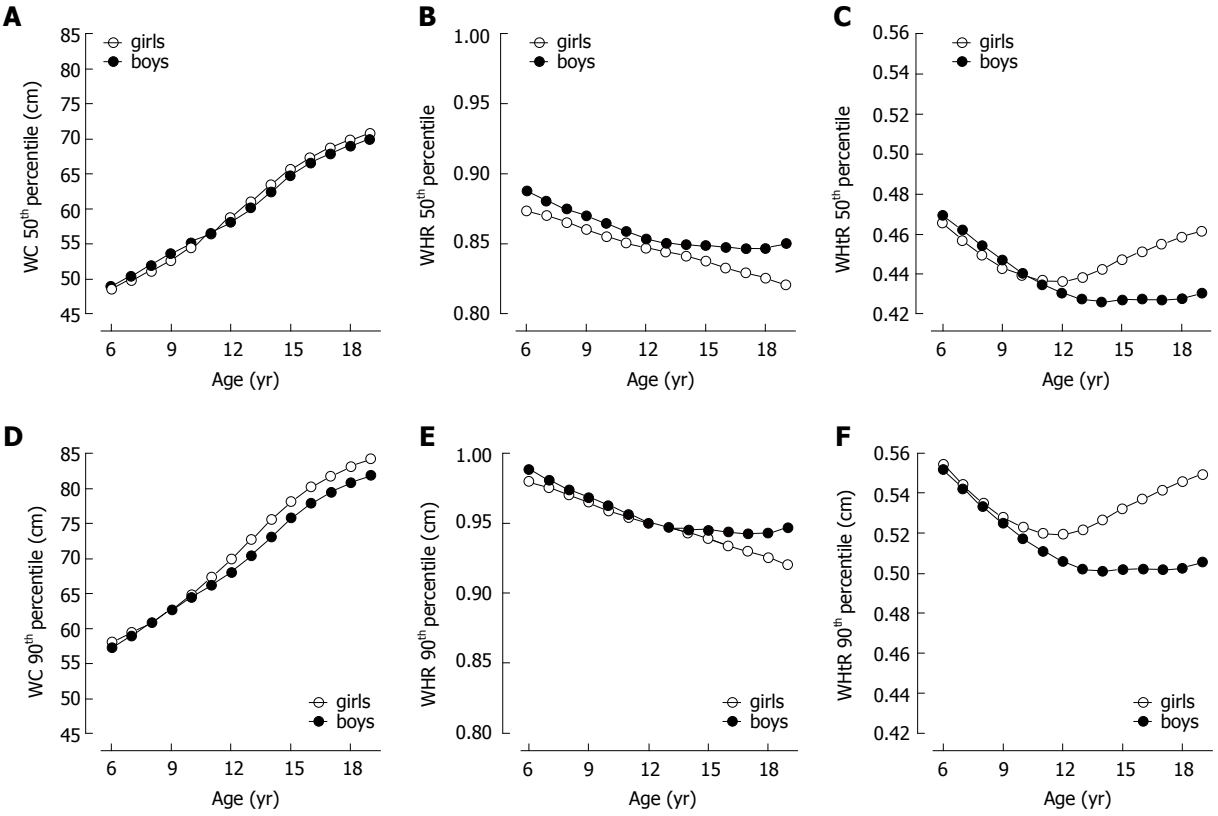



**Figure 1 50th and 90th percentile curves.** Waist circumference (WC, A and D), waist to hip ratio (WHR, B and E), and waist to height ratio (WHtR, C and F) for Yemeni boys (filled circles) and girls (empty circles).

**Table 4 Age, and gender specific smoothed waist-to-height ratio percentiles for Yemeni children and adolescents aged 6 to 19 years**

| Age (yr) | *n* | 3rd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97th |
|---|---|---|---|---|---|---|---|---|---|---|
| Boys | | | | | | | | | | |
| 6 | 47 | 0.35 | 0.37 | 0.4 | 0.44 | 0.47 | 0.51 | 0.55 | 0.59 | 0.61 |
| 7 | 90 | 0.35 | 0.36 | 0.39 | 0.43 | 0.46 | 0.50 | 0.54 | 0.58 | 0.60 |
| 8 | 99 | 0.34 | 0.36 | 0.38 | 0.42 | 0.45 | 0.49 | 0.53 | 0.57 | 0.59 |
| 9 | 101 | 0.34 | 0.35 | 0.38 | 0.41 | 0.45 | 0.48 | 0.53 | 0.56 | 0.58 |
| 10 | 132 | 0.33 | 0.35 | 0.37 | 0.41 | 0.44 | 0.47 | 0.52 | 0.55 | 0.57 |
| 11 | 102 | 0.33 | 0.34 | 0.37 | 0.40 | 0.44 | 0.47 | 0.51 | 0.54 | 0.57 |
| 12 | 157 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.51 | 0.54 | 0.56 |
| 13 | 116 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.56 |
| 14 | 158 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.55 |
| 15 | 112 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.56 |
| 16 | 100 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.56 |
| 17 | 119 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.56 |
| 18 | 133 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.53 | 0.56 |
| 19 | 98 | 0.32 | 0.34 | 0.36 | 0.40 | 0.43 | 0.46 | 0.51 | 0.54 | 0.56 |
| Girls | | | | | | | | | | |
| 6 | 51 | 0.34 | 0.36 | 0.38 | 0.43 | 0.47 | 0.51 | 0.55 | 0.59 | 0.61 |
| 7 | 79 | 0.33 | 0.35 | 0.38 | 0.42 | 0.46 | 0.50 | 0.54 | 0.58 | 0.60 |
| 8 | 86 | 0.32 | 0.34 | 0.37 | 0.41 | 0.45 | 0.49 | 0.53 | 0.57 | 0.59 |
| 9 | 121 | 0.32 | 0.34 | 0.36 | 0.41 | 0.44 | 0.48 | 0.53 | 0.56 | 0.58 |
| 10 | 164 | 0.32 | 0.34 | 0.36 | 0.40 | 0.44 | 0.48 | 0.52 | 0.55 | 0.58 |
| 11 | 123 | 0.32 | 0.33 | 0.36 | 0.40 | 0.44 | 0.48 | 0.52 | 0.55 | 0.57 |
| 12 | 124 | 0.32 | 0.33 | 0.36 | 0.40 | 0.44 | 0.48 | 0.52 | 0.55 | 0.57 |
| 13 | 132 | 0.32 | 0.33 | 0.36 | 0.40 | 0.44 | 0.48 | 0.52 | 0.55 | 0.57 |
| 14 | 130 | 0.32 | 0.34 | 0.36 | 0.40 | 0.44 | 0.48 | 0.53 | 0.56 | 0.58 |
| 15 | 102 | 0.32 | 0.34 | 0.37 | 0.41 | 0.45 | 0.49 | 0.53 | 0.56 | 0.59 |
| 16 | 108 | 0.33 | 0.34 | 0.37 | 0.41 | 0.45 | 0.49 | 0.54 | 0.57 | 0.59 |
| 17 | 97 | 0.33 | 0.35 | 0.37 | 0.42 | 0.45 | 0.50 | 0.54 | 0.57 | 0.60 |
| 18 | 128 | 0.33 | 0.35 | 0.38 | 0.42 | 0.46 | 0.50 | 0.55 | 0.58 | 0.60 |
| 19 | 105 | 0.33 | 0.35 | 0.38 | 0.42 | 0.46 | 0.50 | 0.55 | 0.58 | 0.60 |

Age, and gender specific smoothed waist-to-height ratio percentiles for Yemeni children and adolescents aged 6-19 years ($n$ = 3114).

**Table 5 Logistic regression of factors associated to different definitions of central obesity**

| Variables | WHtR ⩾ 0.5 OR (95%CI) | WC ⩾ 90th OR (95%CI) | WHtR ⩾ 0.5/WC ⩾ 90th OR (95%CI) |
|---|---|---|---|
| Age (yr) | 0.98 (0.94-1.02) | 0.95 (0.90-1.00) | 0.96 (0.90-1.01) |
| Gender (girls *vs* boys) | 1.55 (1.29-1.89) | 0.97 (0.77-1.22) | 1.22 (0.94-1.57) |
| Location (rural *vs* urban) | 0.66 (0.54-0.79) | 0.52 (0.41-0.66) | 0.52 (0.40-0.67) |
| Education (yr) | 0.99 (0.95-1.03) | 1.08 (1.02-1.14) | 1.05 (0.99-1.11) |
| Sedentary (*vs* active) | 1.42 (1.14-1.75) | 1.52 (1.17-1.98) | 1.65 (1.24-2.19) |

Logistic regression of factors associated to different definitions of central obesity: waist-to-height ratio (WHtR) ⩾ 0.5, waist circumference (WC) ⩾ 90th percentile, and the combination of WHtR ⩾ 0.5, and WC ⩾ 90th percentile. Data are reported as adjusted odd ratio (OR) with 95%CI.

and age-specific WC, WHR and WHtR percentiles for Yemeni children 6 to 19 years of age. As observed in other studies WC increases with age, boys having higher WC than girls at childhood[3,4,12,20-24]. In Yemen central obesity is more prevalent in adult women than in men[13] and, according to the present findings, this difference originates at early adolescence when WC starts to be higher in girls than in boys. A WC level of action of 80 cm has been proposed for adult women[25]. The 90th percentile of WC in Yemeni girls crosses this limit already at the age of 16 years. Conversely boys never reach the WC level of action of 94 cm proposed for adult men[25]. According to the present findings the prevalence of central obesity (WC ⩾ 90th percentile, or WHtR ⩾ 0.5, or both WHtR ⩾ 0.5 and WC ⩾ 90th percentile) was higher in urban than in rural settings, and in subjects with sedentary than active lifestyle. Years of school education, which can be considered an index of census, was predictor of having a WC ⩾ 90th. Cut off values of WC have to be based on percentiles to compensate for variation in child development. WHtR was more recently proposed to overcome this limit and to offer the simplest cut-off value for screening central obesity in the clinical practice: "keep your WC to less than half your height"[9]. Also when considering WHtR, the prevalence of central obesity in Yemen was higher among girls than boys, differences being evident after early adolescence. Similarly after early adolescence WHR in boys had a plateau while in girls it

**Table 6 Comparison between waist-to-height ratio 10th, 50th and 90th percentiles for Yemeni, Pakistani (*n* = 12), and Norwegian (*n* = 22) boys and girls**

| Age | Y | P | N | Y | P | N | Y | P | N |
|---|---|---|---|---|---|---|---|---|---|
| | 10th percentiles | | | 50th percentiles | | | 90th percentiles | | |
| Boys | | | | | | | | | |
| 6 | 0.4 | 0.41 | 0.41 | 0.47 | 0.46 | 0.45 | 0.55 | 0.52 | 0.49 |
| 7 | 0.39 | 0.4 | 0.4 | 0.46 | 0.45 | 0.44 | 0.54 | 0.52 | 0.48 |
| 8 | 0.38 | 0.4 | 0.39 | 0.45 | 0.45 | 0.43 | 0.53 | 0.52 | 0.48 |
| 9 | 0.38 | 0.39 | 0.38 | 0.45 | 0.45 | 0.42 | 0.53 | 0.52 | 0.48 |
| 10 | 0.37 | 0.39 | 0.38 | 0.44 | 0.44 | 0.42 | 0.52 | 0.52 | 0.48 |
| 11 | 0.37 | 0.38 | 0.37 | 0.44 | 0.44 | 0.42 | 0.51 | 0.52 | 0.48 |
| 12 | 0.36 | 0.38 | 0.37 | 0.43 | 0.44 | 0.41 | 0.51 | 0.52 | 0.47 |
| 13 | 0.36 | - | 0.36 | 0.43 | - | 0.41 | 0.5 | - | 0.47 |
| 14 | 0.36 | - | 0.36 | 0.43 | - | 0.41 | 0.5 | - | 0.46 |
| 15 | 0.36 | - | 0.37 | 0.43 | - | 0.41 | 0.5 | - | 0.46 |
| 16 | 0.36 | - | 0.37 | 0.43 | - | 0.41 | 0.5 | - | 0.47 |
| 17 | 0.36 | - | 0.38 | 0.43 | - | 0.42 | 0.5 | - | 0.47 |
| 18 | 0.36 | - | 0.38 | 0.43 | - | 0.43 | 0.5 | - | 0.48 |
| 19 | 0.36 | - | - | 0.43 | - | - | 0.51 | - | - |
| Girls | | | | | | | | | |
| 6 | 0.38 | 0.41 | 0.42 | 0.47 | 0.45 | 0.45 | 0.55 | 0.51 | 0.49 |
| 7 | 0.38 | 0.4 | 0.4 | 0.46 | 0.45 | 0.43 | 0.54 | 0.52 | 0.49 |
| 8 | 0.37 | 0.4 | 0.4 | 0.45 | 0.45 | 0.43 | 0.53 | 0.52 | 0.48 |
| 9 | 0.36 | 0.4 | 0.39 | 0.44 | 0.45 | 0.42 | 0.53 | 0.53 | 0.48 |
| 10 | 0.36 | 0.39 | 0.39 | 0.44 | 0.44 | 0.41 | 0.52 | 0.53 | 0.47 |
| 11 | 0.36 | 0.38 | 0.38 | 0.44 | 0.44 | 0.41 | 0.52 | 0.53 | 0.46 |
| 12 | 0.36 | 0.37 | 0.38 | 0.44 | 0.43 | 0.4 | 0.52 | 0.52 | 0.46 |
| 13 | 0.36 | - | 0.37 | 0.44 | - | 0.4 | 0.52 | - | 0.45 |
| 14 | 0.36 | - | 0.37 | 0.44 | - | 0.4 | 0.53 | - | 0.45 |
| 15 | 0.37 | - | 0.37 | 0.45 | - | 0.4 | 0.53 | - | 0.45 |
| 16 | 0.37 | - | 0.38 | 0.45 | - | 0.4 | 0.54 | - | 0.45 |
| 17 | 0.37 | - | 0.38 | 0.45 | - | 0.41 | 0.54 | - | 0.46 |
| 18 | 0.38 | - | 0.39 | 0.46 | - | 0.42 | 0.55 | - | 0.47 |
| 19 | 0.38 | - | - | 0.46 | - | - | 0.55 | - | - |

Comparison between waist-to-height ratio 10th, 50th and 90th percentiles for Yemeni (Y), Pakistani (P), and Norwegian (N) boys and girls.

continued to decrease. The clear identification of a high risk subgroup is important. According to the present survey girls at early adolescence, living in urban areas, educated, with sedentary lifestyle have to be considered as a target for future educational programs aimed at limiting central obesity in Yemeni adult women. Furthermore central obesity in children was reported to be an independent predictor of insulin resistance, lipid levels, and high blood pressure[6,26]. As suggested by the international diabetes foundation[27] outcome studies investigating future metabolic syndrome, diabetes and cardiovascular disease in developing countries are required.

During the last decades, age and sex-specific WC percentiles have been obtained in different western, and MEC countries. In Yemen early adolescence is the starting point for the increase of the 90th percentile of WC in girls differently from what observed in Norway[23] where at this age the same increase was reported to occur in boys (Figure 2). A low physical activity for girls living in Yemen might be considered. This age related change was however not reported in Turkish girls[21]. The differences among females in Yemen may be related to other factors such as weather, absence of sidewalks and parks, and culture such as attitudes of males towards women walking for leisure. Other cultural aspects, such as the adoption of western aesthetic values modifying the traditional consideration of plumpness as an index of beauty might also play a role[28]. In Kuwait[3] early adolescence is the starting point for central obesity both in girls and in boys probably because of the high income of the country (Figure 2). Comparison with data obtained in United States[22] might be more complex due to the composite ethnicity of participants.

When considering WHtR data from different countries, the average height in the country has to be considered. In Yemen the 90th percentile values were comparable to those found in Pakistan[12] although significantly higher than those found in Norway[23] (Table 6). More precisely, when considering this index in developing countries the possible contribution of malnutrition on height should be taken into account. According to the present findings more than 50% of Yemeni girls and boys aged 6 to 18 years have WC values below those of the United States 10th percentiles[22] (Figure 2). Even when considering subjects living in the MEC, more than 25% of Yemeni children have WC values below those of the 10th percentiles measured in Kuwait[3] and Pakistan[12] (Figure 2).

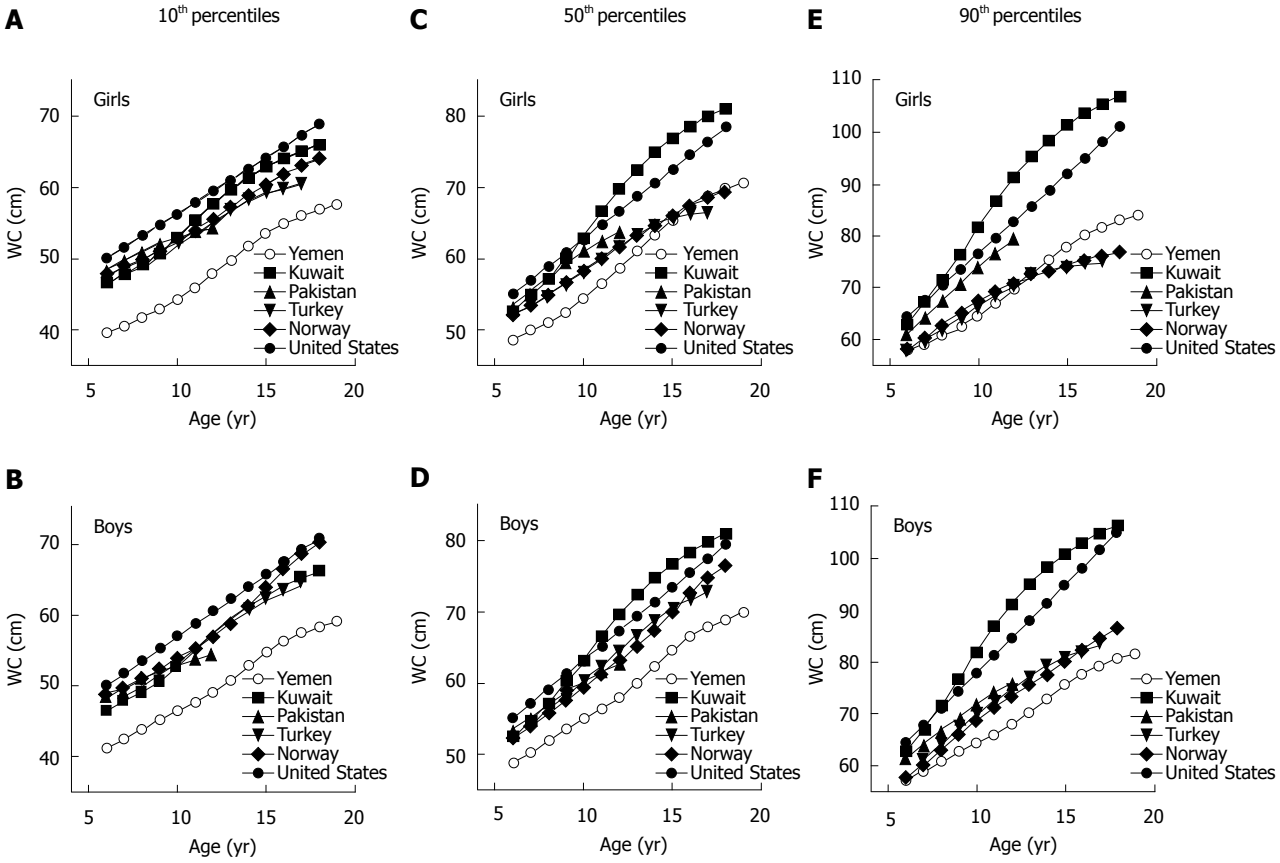



**Figure 2 Comparison of waist circumference 10th, 50th, and 90th percentile curve.** Waist circumference (WC) 10th, 50th, and 90th percentile curves for Yemeni, Kuwaiti[3], Pakistani[12], Turkish[20], United States[21], and Norwegian[22] girls (A, C, E) and boys (B, D, F).

There are limitations and strengths in this study. A first limitation is the unequal number of subjects in the 28 age strata. The population sample in the HYDY study was stratified by decades of age rather than by years of age. However discrepancy between age groups is limited and the less numerous group included an acceptable number of subjects. Secondly, the Tanner stage was not assessed. A third limitation is that in the HYDY study biochemical investigations on blood (glucose, cholesterol, and triglycerides) were not assessed in subjects aged less than 14 years. This decision, which precluded us drawing conclusions about the associations with obesity indices, was taken considering the child's perceptions and fears of the procedure of blood collection. The strengths of our study are (1) the novelty in Yemeni children; (2) the large sample size of children studied over different areas of the country with a constantly reproducible study procedure; and finally (3) the door to door approach adopted in the sampling procedure. We decided to adopt a door-to-door procedure rather than performing a school-based study because in low income countries not all children may have access to school. Considering the very high response rate we can exclude the presence of relevant selection bias. Our data provide the first description of the percentile distribution, derived from a large nationally representative sample of 6-19 year old children, of WC, WHR and WHtR in urban and rural Yemeni population.

In conclusion, HYDY data show a large discrepancy of central obesity prevalence among Yemeni children probably because the country is still in an early stage of the nutritional transition and there are population segments which are affected by malnutrition[29]. Prevalence of central obesity in Yemeni children is low, being associated with urbanization and sedentary lifestyle. WC, WHtR and WHR changed similarly in girls and boys until early adolescence. Thereafter, differently from what observed in Western countries, WC, WHtR and WHR changes increased more in girls than in boys. The importance of changes observed at early adolescence among girls living in urban areas, might be relevant for future National programs aimed at promoting physical activity and control of central obesity in women.

## ACKNOWLEDGMENTS

We thank the many enthusiastic workers in Yemen who have contributed to the study: Capital area: Hisham Abdulrab Ali Al Qubati, Raed Faisal Nasser, Aziz Hussain Ali Moshoeh, Mohammed Abdulah Ashmeni, Abdulhamed Hulfy Alanashany, Mohammed Ibrahim AhmedAl Hamali, Naif Radman Hamdan Agial, Sami Abduh Kaid Ahmed Alwessaby, Munera Abduwhab Saleh Yahya, Hyam Abdul Kareem Al Hyfi, Ziad Abdullah Mohammed Al Mass, Liza Nayib Shamiri, Sahar Ahmed Abdul-

lah Moqbel, Abdullah Assan Mohammed Guly, Abdulaziz Saeed Salem Bawazir, Abi Saleh Al Rawi, Kaled Sadeg Ali Al-Shaibari, Abdul Bari Mahfoudh Omar Al Daba, Osama Hadi Abdu Haig, Gamil Al Hamdi Abdo Mohsan; Inland area: Khalil Abdulwahid Ali Al-Kuhlani, Fahd Ahmed Ahmed Ali Al Durafi, Moad Ahmed Abdul Al Karem Al Shorihy, Ashra F Saheed Abdalh, Omar Ahmed Abdul Aziz, Kalil Abdu Mohammed Assufi, Ahmed Saeed Ahmed Al Terin, Mamon Ahdo Alrahmen Alp, Fuad Mohammed Ali Abdu Ghaleb, Mohammed Ali Hussen, Mobark Mohammad Dabwan Quasem, Awad Hamad Mohammad; Coastal area: Yasin Mohammad Al Hay Ibrahim, Abdu Al Aleem Qaid Abdu Al Aleem Safian, Mohammed Ali Mohammed Salman, Abdh Kozim Abdullah Al Nhozy, Mohammed Awad Omer Hydhi, Hussein Omar Al Ahbari, Adel Hai Bin Obedellah, Khalid Hussan Khaleby. HYDY Study Group Steering Committee: Pietro Amedeo Modesti (Chair), Husni Al Goshae, Mohamed Bamoshmoosh, Dawood Al-Hidabi, Gian Franco Gensini, Marzia Baldereschi, Stefano Rapi, Luciano Massetti. Monitoring Board: Mohamed Shamsuddin, Hameed M Aklan, Mahdy Al-Karewany, Abdul-Malik Al-Amrany, Ferdawsi Al-Jilani; Data Center: Ammar Al-Ghadi, Giulia Elisa Cambi, Antonella Ferrari.

## COMMENTS

### Background

Children obesity also in the Middle Crescent Area is a matter of growing concern. Childhood central obesity is assessed by measuring waist circumference (WC). However, ethnicity and environmental differences might influence body proportions, and the usefulness of national references to control for variations between populations was suggested.

### Research frontiers

In children WC has to be expressed relatively to their sex and age peers. Differently from WC and waist to hip ratio (WHR) a recently proposed index, waist-to-height ratio (WHtR), is only weakly associated with age and gender, and different studies are suggesting a single cut-off value for defining central obesity (WHtR $\geqslant$ 0.5).

### Innovations and breakthroughs

Prevalence of central obesity in Yemeni children is low, being associated with urbanization and sedentary lifestyle. WC, WHtR and WHR changed similarly in girls and boys until early adolescence. Thereafter, differently from what observed in Western countries, WC, WHtR and WHR changes increased more in girls than in boys.

### Applications

The importance of the changes observed at early adolescence among sedentary girls living in urban areas, might be relevant for future National program aimed at promoting physical activity and control of central obesity in women.

### Terminology

Central obesity measures the abdominal obesity using parameters such as WC, WHR and WHtR, and seems to be better correlated with cardiovascular disease and mortality than general obesity measured using the body mass index.

### Peer review

The authors provided the first central obesity percentile curves of WC, WHtR and WHR for Yemeni children aged six to nineteen years. As expected the prevalence of central obesity in Yemeni children is low, being associated with urbanization and sedentary lifestyle. The study showed also that WC, WHtR and WHR values changed similarly in girls and boys until early adolescence. However, thereafter, differently from what observed in Western countries, these changes increased more in girls than in boys indicating that this is a crucial moment in the arising of adult women central obesity.

## REFERENCES


1 **Han JC**, Lawlor DA, Kimm SY. Childhood obesity. *Lancet* 2010; **375**: 1737-1748 [PMID: 20451244 DOI: 10.1016/S0140-6736(10)60171-7]

2 **Ng SW**, Zaghloul S, Ali HI, Harrison G, Popkin BM. The prevalence and trends of overweight, obesity and nutrition-related non-communicable diseases in the Arabian Gulf States. *Obes Rev* 2011; **12**: 1-13 [PMID: 20546144 DOI: 10.1111/j.1467-789X.2010.00750.x]

3 **Jackson RT**, Al Hamad N, Prakash P, Al Somaie M. Waist circumference percentiles for Kuwaiti children and adolescents. *Public Health Nutr* 2011; **14**: 70-76 [PMID: 20920388 DOI: 10.1017/S1368980010002600]

4 **Kelishadi R**, Gouya MM, Ardalan G, Hosseini M, Motaghian M, Delavari A, Majdzadeh R, Heidarzadeh A, Mahmoud-Arabi MS, Riazi MM. First reference curves of waist and hip circumferences in an Asian population of youths: CASPIAN study. *J Trop Pediatr* 2007; **53**: 158-164 [PMID: 17308326 DOI: 10.1093/tropej/fml090]

5 **Kahn HS**, Imperatore G, Cheng YJ. A population-based comparison of BMI percentiles and waist-to-height ratio for identifying cardiovascular risk in youth. *J Pediatr* 2005; **146**: 482-488 [PMID: 15812450 DOI: 10.1016/j.jpeds.2004.12.028]

6 **Freedman DS**, Kahn HS, Mei Z, Grummer-Strawn LM, Dietz WH, Srinivasan SR, Berenson GS. Relation of body mass index and waist-to-height ratio to cardiovascular disease risk factors in children and adolescents: the Bogalusa Heart Study. *Am J Clin Nutr* 2007; **86**: 33-40 [PMID: 17616760]

7 **Savva SC**, Kourides Y, Tornaritis M, Epiphaniou-Savva M, Tafouna P, Kafatos A. Reference growth curves for cypriot children 6 to 17 years of age. *Obes Res* 2001; **9**: 754-762 [PMID: 11743059 DOI: 10.1038/oby.2001.104]

8 **Hara M**, Saitou E, Iwata F, Okada T, Harada K. Waist-to-height ratio is the best predictor of cardiovascular disease risk factors in Japanese schoolchildren. *J Atheroscler Thromb* 2002; **9**: 127-132 [PMID: 12226553 DOI: 10.5551/jat.9.127]

9 **McCarthy HD**, Ashwell M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message--'keep your waist circumference to less than half your height'. *Int J Obes* (Lond) 2006; **30**: 988-992 [PMID: 16432546 DOI: 10.1038/sj.ijo.0803226]

10 **Roswall J**, Bergman S, Almqvist-Tangen G, Alm B, Niklasson A, Nierop AF, Dahlgren J. Population-based waist circumference and waist-to-height ratio reference values in preschool children. *Acta Paediatr* 2009; **98**: 1632-1636 [PMID: 19604174 DOI: 10.1111/j.1651-2227.2009.01430.x]

11 **Motlagh B**, O'Donnell M, Yusuf S. Prevalence of cardiovascular risk factors in the Middle East: a systematic review. *Eur J Cardiovasc Prev Rehabil* 2009; **16**: 268-280 [PMID: 19398913 DOI: 10.1097/HJR.0b013e328322ca1b]

12 **Mushtaq MU**, Gull S, Abdullah HM, Shahid U, Shad MA, Akram J. Waist circumference, waist-hip ratio and waist-height ratio percentiles and central obesity among Pakistani children aged five to twelve years. *BMC Pediatr* 2011; **11**: 105 [PMID: 22104025 DOI: 10.1186/1471-2431-11-105]

13 **Modesti PA**, Rapi S, Bamoshmoosh M, Baldereschi M, Massetti L, Padeletti L, Gensini GF, Zhao D, Al-Hidabi D, Al Goshae H. Impact of one or two visits strategy on hypertension burden estimation in HYDY, a population-based cross-sectional study: implications for healthcare resource allocation decision making. *BMJ Open* 2012; **2**: e001062[PMID: 22874627]

14 US Census Bureau: International Data Base (IDB).Yemen 2008. Available from: URL: http: //www.census.gov/ipc/www/idb/groups.php

15 **Cole TJ**, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity world-

wide: international survey. *BMJ* 2000; **320**: 1240-1243 [PMID: 10797032 DOI: 10.1136/bmj.320.7244.1240]
16 **Modesti PA**, Massetti L, Bamoshmoosh M, Baldereschi M, Cambi GE, Rapi S. The impact of fine-tuning of optical recognition system on database reliability. *Comput Biol Med* 2012; **42**: 778-783 [PMID: 22658683 DOI: 10.1016/j.compbiomed.2012.05.003]
17 **Cole TJ**, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 1998; **17**: 407-429 [PMID: 9496720 DOI: 10.1002/(SICI)1097-0258(19980228)17]
18 **Cole TJ**, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992; **11**: 1305-1319 [PMID: 1518992 DOI: 10.1002/sim.4780111005]
19 **Rigby RA**, Stasinopoulos DM. Smooth centile curves for skew and kurtotic data modelled using the Box-Cox power exponential distribution. *Stat Med* 2004; **23**: 3053-3076 [PMID: 15351960 DOI: 10.1002/sim.1861]
20 **Maffeis C**, Pietrobelli A, Grezzani A, Provera S, Tatò L. Waist circumference and cardiovascular risk factors in prepubertal children. *Obes Res* 2001; **9**: 179-187 [PMID: 11323443 DOI: 10.1038/oby.2001.19]
21 **Hatipoglu N**, Ozturk A, Mazicioglu MM, Kurtoglu S, Seyhan S, Lokoglu F. Waist circumference percentiles for 7- to 17-year-old Turkish children and adolescents. *Eur J Pediatr* 2008; **167**: 383-389 [PMID: 17487506 DOI: 10.1007/s00431-007-0502-3]
22 **Fernández JR**, Redden DT, Pietrobelli A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr* 2004; **145**: 439-444 [PMID: 15480363 DOI: 10.1016/j.jpeds.2004.06.044]
23 **Brannsether B**, Roelants M, Bjerknes R, Júlíusson PB. Waist circumference and waist-to-height ratio in Norwegian children 4-18 years of age: reference values and cut-off levels. *Acta Paediatr* 2011; **100**: 1576-1582 [PMID: 21627692 DOI: 10.1111/j.1651-2227.2011.02370.x]
24 **Ozaki R**, Qiao Q, Wong GW, Chan MH, So WY, Tong PC, Ho CS, Ko GT, Kong AP, Lam CW, Tuomilehto J, Chan JC. Overweight, family history of diabetes and attending schools of lower academic grading are independent predictors for metabolic syndrome in Hong Kong Chinese adolescents. *Arch Dis Child* 2007; **92**: 224-228 [PMID: 17088339 DOI: 10.1136/adc.2006.100453]
25 **Lean ME**, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. *BMJ* 1995; **311**: 158-161 [PMID: 7613427 DOI: 10.1136/bmj.311.6998.158]
26 **Lee S**, Bacha F, Arslanian SA. Waist circumference, blood pressure, and lipid components of the metabolic syndrome. *J Pediatr* 2006; **149**: 809-816 [PMID: 17137898 DOI: 10.1016/j.jpeds.2006.08.075]
27 **Zimmet P**, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, Wong G, Bennett P, Shaw J, Caprio S. The metabolic syndrome in children and adolescents. *Lancet* 2007; **369**: 2059-2061 [PMID: 17586288 DOI: 10.1016/S0140-6736(07)60958-1]
28 **Al-Sendi AM**, Shetty P, Musaiger AO. Body weight perception among Bahraini adolescents. *Child Care Health Dev* 2004; **30**: 369-376 [PMID: 15191428 DOI: 10.1111/j.1365-2214.2004.00425.x]
29 **Burki TK.** Yemen's hunger crisis. *Lancet* 2012; **380**: 637-638 [PMID: 22908378 DOI: 10.1016/S0140-6736(12)61356-7]

**P- Reviewers** Merchant A, Rani R **S- Editor** Gou SX
**L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.305

*World J Cardiol* 2013 August 26; 5(8): 305-312
ISSN 1949-8462 (online)


BRIEF ARTICLE

# Blood cellular mutant LXR-α protein stability governs initiation of coronary heart disease

Mansi Arora, Deepak Kaul, Yash Paul Sharma

Mansi Arora, Deepak Kaul, Yash Paul Sharma, Department of Experimental Medicine, Biotechnology and Cardiology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
Author contributions: Kaul D designed the study; Arora M executed the study; Sharma YP provided the blood samples.
Supported by Indian Council of Medical Research, New Delhi, India
Correspondence to: Deepak Kaul, PhD, Professor and Head, Departments of Experimental Medicine, Biotechnology and Cardiology, Post Graduate Institute of Medical Education and Research, Pin-133001, Chandigarh 160012, India. dkaul-24@hotmail.com
Telephone: +91-172-2755228 Fax: +91-172-2744401
Received: March 3, 2013 Revised: June 3, 2013
Accepted: July 18, 2013
Published online: August 26, 2013

## Abstract

**AIM:** To investigate the role of [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1 (BARD1)]/BRCA1 E3-ubiquitin ligase complex in governing the stability of mutant liver X receptor-α (LXR-α) protein in coronary heart disease (CHD) subjects.

**METHODS:** The expression analysis of various genes was carried out by quantitative real time polymerase chain reaction and western blotting within blood mononuclear cells of human CHD subjects at various stages of coronary occlusion and their corresponding normal healthy counterparts. Immunoprecipitation experiments were performed to establish protein interactions between LXR-α and BARD1. Peripheral blood mononuclear cells were cultured and exposed to Vitamin $D_3$ and Cisplatin to validate the degradation of mutant LXR-α protein in CHD subjects by BARD1/BRCA1 complex.

**RESULTS:** The expression of mutant LXR-α protein in CHD subjects was found to decrease gradually with the severity of coronary occlusion exhibiting a strong negative correlation, $r$ = -0.975 at $P < 0.001$. Further, the expression of BARD1 and BRCA1 also increased with the disease severity, $r$ = 0.895 and 0.873 respectively ($P < 0.001$). Immunoprecipitation studies established that BARD1/BRCA1 complex degrades mutant LXR-α *via* ubiquitination. The absence of functional LXR-α protein resulted in increased expression of inflammatory cytokines such as interleukin (IL)-6, IL-8 and interferon-γ and decreased expression of ABCA1 (ATP-binding cassette A1) ($r$ = 0.932, 0.949, 0.918 and -0.902 with respect to Gensini score; $P < 0.001$). Additionally, cell culture experiments proved that Vitamin $D_3$ could prevent the degradation of mutant LXR-α and restore its functional activity to some extent.

**CONCLUSION:** Mutant LXR-α protein in CHD subjects is degraded by BARD1/BRCA1 complex and Vitamin $D_3$ can rescue and restore its function.



**Key words:** Mutant liver X receptor-α; Ubiquitination; Breast and ovarian cancer susceptibility 1-associated RING domain 1/breast and ovarian cancer susceptibility 1; Mononuclear Cells; Coronary heart disease subjects; Vitamin $D_3$

**Core tip:** The present study proposes that the stability of mutant liver X receptor-α (LXR-α) protein in blood mononuclear cells of human coronary heart disease (CHD) subjects is governed by its ubiquitination dependent degradation by [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain1 (BARD1)]/BRCA1 E3 ubiquitin ligase complex. Additionally, BARD1/BRCA1 expression shows an increasing trend with respect to severity of coronary occlusion. This degradation is rescued to some extent by the ability of Vitamin $D_3$ to bind mutant LXR-α protein thus providing warranted evidence that dietary supplementation of Vitamin $D_3$ in such subjects may be exploited therapeutically.

Arora M, Kaul D, Sharma YP. Blood cellular mutant LXR-α protein stability governs initiation of coronary heart disease. *World J Cardiol* 2013; 5(8): 305-312 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/305.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.305

## INTRODUCTION

Liver X receptor-α (LXR-α) is a ligand activated transcription factor that plays a pivotal athero-protective role by regulating genes involved in lipid metabolism and reverse cholesterol transport [*e.g.,* ATP-binding cassette A1 (ABCA1), ABCG1, Apolipoprotein E] and by inhibiting nuclear factor kappa-B mediated inflammatory responses and proliferation of vascular smooth muscle cells[1-7]. Several *in vitro* and *in vivo* studies in animal models of atherosclerosis have shown that LXR-α agonists can attenuate lesion progression and also lead to regression of an already established plaque[8-14]. The observation that statins as well as vitamin C, both have an inherent ability to upregulate LXR-α[15] further underline its importance. Findings from our laboratory have demonstrated that both normolipidemic and hyperlipidemic human coronary heart disease (CHD) subjects have significantly higher expression of blood cellular LXR-α as compared to the corresponding controls[16]. This is in sharp contrast with the observed protective role of LXR-α. Paradoxically there is an increased expression of LXR-α with the corresponding increase in severity of coronary occlusion[17]. Further work has partly resolved this paradox by revealing three critical mutations in its ligand binding domain involving Asp324, Pro327 and Arg328 which compromises its ability to interact and get activated by its natural ligands[17]. But to fully understand this apparent paradox it is imperative to explore the stability and expression of this mutant LXR-α protein. Recently Kim *et al*[19] have shown that ligand free LXR-α interacts with an E3 ubiquitin ligase heterodimer complex of breast and ovarian cancer susceptibility 1 (BRCA1) and BRCA1-associated RING domain 1 (BARD1), and is subsequently degraded. Since mutant LXR-α in CHD patients is also unable to bind to its ligand, the present study was addressed to explore the role of BARD1/BRCA1 (breast and ovarian cancer susceptibility 1) complex in governing the stability of mutant LXR-α in these subjects.

## MATERIALS AND METHODS

### Subject selection

Freshly diagnosed male subjects ($n$ = 40) with confirmed coronary heart disease (diagnosed for the first time upon coronary angiography) and control subjects ($n$ = 10, age and gender matched with angiographically proven normal coronary arteries) were selected for the study from the outpatient clinic of Department of Cardiology, Postgraduate Institute of Medical Education and Research, Chandigarh, with their prior informed consent. Females, diabetics, individuals suffering from cardiomyopathies, any infectious disease, systemic illness, serious organ disease, serious psychiatric illness, chronic alcohol abuse and anti-convulsant therapy were excluded from this study. Further, subjects taking any drug namely lipid lowering drugs or antihypertensive or anti-diabetic drugs (which could interfere with the study) were also excluded from the study. The study was approved by institute's ethical committee and conforms to the principles outlined in the declaration of Helsinki[19]. The laboratory variables of the patients are given in Table 1. The severity of coronary occlusion in CHD patients was measured by Gensini Score[20] and the subjects were categorized into five groups as described in Table 2.

### Gene expression analysis and immunoprecipitation

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 mL of heparinized blood using Ficoll-Hypaque density gradient method[21]. RNA was isolated using standard guanidinium thiocynate method[22]. The extracted RNA was reverse transcribed using Revert Aid™ first strand synthe 2.3). β-actin (Sigma Aldrich) was taken as an invariant control for both transcriptional and translational expression studies.

### Immunoprecipitation and western blotting

The cells were lysed with non-denaturing lysis buffer [ 20 mmol/L Tris HCl (pH = 8), 137 mmol/L NaCl, 10% glycerol, 1% Triton X-100 and 2 mmol/L EDTA (Ethylene Diamine Tetraacetic Acid)] containing protease inhibitor cocktail (Sigma Aldrich). For immunoprecipitation, equal amounts of total cell extracts from CHD subjects (GS = 10-20) and healthy controls were incubated with LXR-α antibody, and the immunoprecipitated complexes were collected using protein-G sepharose beads (Sigma Aldrich). Further, pellets were washed 3 times with 1 mL non-denaturing lysis buffer, protein eluted in sample buffer (0.125 mol/L Tris, 2%SDS, 5% 2-mercaptoethanol) and subjected to western blotting. For direct western blotting, protein extracts were electrophoresed by SDS-PAGE ( 125 mL/L, Sodium dodecyl sulphate-poly-acrylamide gel electrophoresis), transferred to nitrocellulose membranes and probed using specific antibodies against LXR-α, BARD1, BRCA1 and β-actin. β-actin was used as an invariant control. Each band on the immunoblot was scanned densitometrically using Scion Image Analysis software. The results were expressed as intensity ratio of target protein to β-actin protein taken as arbitrary unit (AU).

### Cell culture experiments

PBMCs from healthy subjects and CHD subjects were seeded in RPMI 1640 medium containing 10% FCS at 37 ℃ in 5%$CO_2$ atmosphere and exposed to Vitamin $D_3$ ( 1 μmol/L) or Cisplatin (30 μmol/L) for 36 h. After the incubation period, cells were harvested and processed for RNA and protein isolation by employing standard methods[22,23].

**Table 1 Laboratory variables of subjects employed in the study**

| | Control ($n$ = 10) | CHD subjects ($n$ = 40) | $P$ value |
|---|---|---|---|
| Age (yr) | 51 ± 8 | 53 ± 4 | NS |
| Sex | M | M | NA |
| TC (mg/dL) | 179.5 ± 4.7 | 182.0 ± 3.6 | NS |
| TG (mg/dL) | 156.2 ± 6.3 | 167.1 ± 8.7 | NS |
| HDL-C (mg/dL) | 49.8 ± 1.3 | 42.6 ± 3.9 | NS |
| LDL-C (mg/dL) | 93.24 ± 5.67 | 102.0 ± 5.3 | NS |
| Serum CRP (mg/dL) | 0.63 ± 0.32 | 1.10 ± 0.51 | NS |
| Serum 25 (OH) Vitamin $D_3$ (ng/mL) | 16 ± 2.3 | 7.3 ± 3.2 | S |

CHD: Coronary heart disease; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; M: Male; NS: Not significant; NA: Not applicable; S: Significant; CRP: C-reactive protein.

**Table 2 Subject groups formed on the basis of severity of coronary occlusion as measured by gensini score**

| Group | Gensini score | No. of subjects |
|---|---|---|
| Control | 0 | 10 |
| Group Ⅰ | 1-10 | 10 |
| Group Ⅱ | 11-20 | 10 |
| Group Ⅲ | 21-30 | 10 |
| Group Ⅳ | > 30 | 10 |

### *Statistical analysis*

Statistical analyses were performed by SPSS Windows version 19. Correlation between severity of CHD and expression of various genes was evaluated by Spearman rank-correlation coefficient, $P$ value < 0.01 taken as statistically significant. Data were presented as mean ± SD. Statistical comparisons between multiple groups were made by ANOVA (One way Analysis of Variance). $P$ value < 0.05 was considered statistically significant.

## RESULTS

As reported earlier[17], we observed similar pattern of 3 critical mutations in the ligand binding domain and increased transcriptional expression of LXR-α with respect to increasing coronary occlusion (data not shown) in all subjects employed in this study. In contrast, LXR-α protein expression was found to decrease with increasing severity of coronary occlusion and exhibited a strong negative correlation with gensini score (Figure 1A and B). Correspondingly, the expression of ubiquitin ligase heterodimer BARD1/BRCA1 increased with respect to increasing severity of coronary atherosclerosis (Figures 1C and 2E), showing a strong positive correlation with gensini score (Figure 3A and B). Consequently, the expression of inflammatory genes such as interleukin (IL)-6, IL-8 and interferon (IFN)-γ was found to increase and ABCA1, a direct target of LXR-α responsible for cholesterol efflux, decreased with increasing severity of disease (Figures 1D and 3C-F). Our previous studies have proved that Vitamin $D_3$ can bind to mutant LXR-α ligand binding domain[23]. So, in order to confirm that inability of LXR-α protein to bind to its natural ligands is responsible for its degradation, we exposed patient cells to Vitamin $D_3$ (1 μmol/L). The significant increase in the expression of LXR-α protein in these patient cells unambiguously revealed that Vitamin $D_3$ bound LXR-α is resistant to degradation by BARD1/BRCA1 complex (Figure 2A). Further, though the expression of BARD1/BRCA1 complex in patient PBMCs decreased upon Vitamin $D_3$ exposure, the difference was not significant as compared to patient cells alone (Figure 2B). Also, the significantly increased expression of ABCA1 in patient cells exposed to Vitamin $D_3$ further validated our previous findings[23] that Vitamin $D_3$ is able to restore the functional activity of mutant LXR-α to some extent (Figure 2B). As expected, Vitamin $D_3$ treatment did not have any significant effect on LXR-α protein expression in normal cells which harbor wild type LXR-α (Figure 2A). To ascertain the role of BARD1/BRCA1 dependent ubiquitination in the degradation of mutant ligand-free LXR-α protein, we examined their interaction in patient PBMCs by co-immunoprecipitation assays. CHD subjects with GS = 10-20 were selected as per statistical analysis since such subjects have appreciable expression of both LXR-α and BARD1/BRCA1 complex. Total cell lysates from PBMCs derived from CHD subjects and normal healthy individuals were immune-precipitated with anti LXR-α antibody and immune-blotted with anti-BARD1 antibody. The results revealed that the mutant LXR-α protein strongly associated with BARD1 in CHD subjects as compared to that in healthy controls (Figure 2E). Additionally, direct western blotting also demonstrated the increased expression of BARD1 and BRCA1 protein in PBMCs of CHD subjects as compared to their healthy counterparts (Figure 2E). To further precipitate the role of BARD1/BRCA1 complex in degradation of ligand-free LXR-α in CHD subjects, patient PBMCs were exposed *in vitro* to cisplatin (30 μmol/L) which has recently been shown to inhibit the E3 ubiquitin ligase activity of BARD1/BRCA1 heterodimer[24]. The observed increase in the expression of LXR-α protein in patient cells treated with cisplatin in the absence of Vitamin $D_3$ undoubtedly established the role of BARD1/BRCA1

**Table 3 Primers sequences employed for transcriptional expression analysis of various genes**

| No. | Gene | Primer pair |
|---|---|---|
| 1 | *ABCA1* | Forward: 5′-ACCTCGGGCACCGCCTACAT-3′<br>Reverse: 5′-CGAAGGCCCGCCTGTTTCGT-3′ |
| 2 | *BARD1* | Forward: 5′-GCCAAAGCTGTTTGATGGAT-3′<br>Reverse: 5′-CGAACCCTCTCTGGGTGATA-3′ |
| 3 | *BRCA1* | Forward: 5′-TAGGGCTGGAAGCACAGAGT-3′<br>Reverse: 5′-AATTTCCTCCCCAATGTTCC-3′ |
| 4 | *IFN-γ* | Forward: 5′-CGTTTTGGGTTCTCTTGGCTGTT-3′<br>Reverse: 5′-CTCCTTTTTCGCTTCCCTGTTTT-3′ |
| 5 | *IL-6* | Forward: 5′-TGGGCACAGAACTTATGTTG-3′<br>Reverse: 5′-TTGAGGTAAGCCTACACTTTCC-3′ |
| 6 | *IL-8* | Forward: 5′-ATGACTTCCAAGCTGGCCGTGGCT-3′<br>Reverse: 5′-TCTCAGCCCTCTTCAAAAACTTCT-3′ |
| 7 | *β-actin* | Forward: 5′-CATGTACGTTGCTATCCAGGC-3′<br>Reverse: 5′-CTCCTTAATGTCACGCACGAT-3′ |

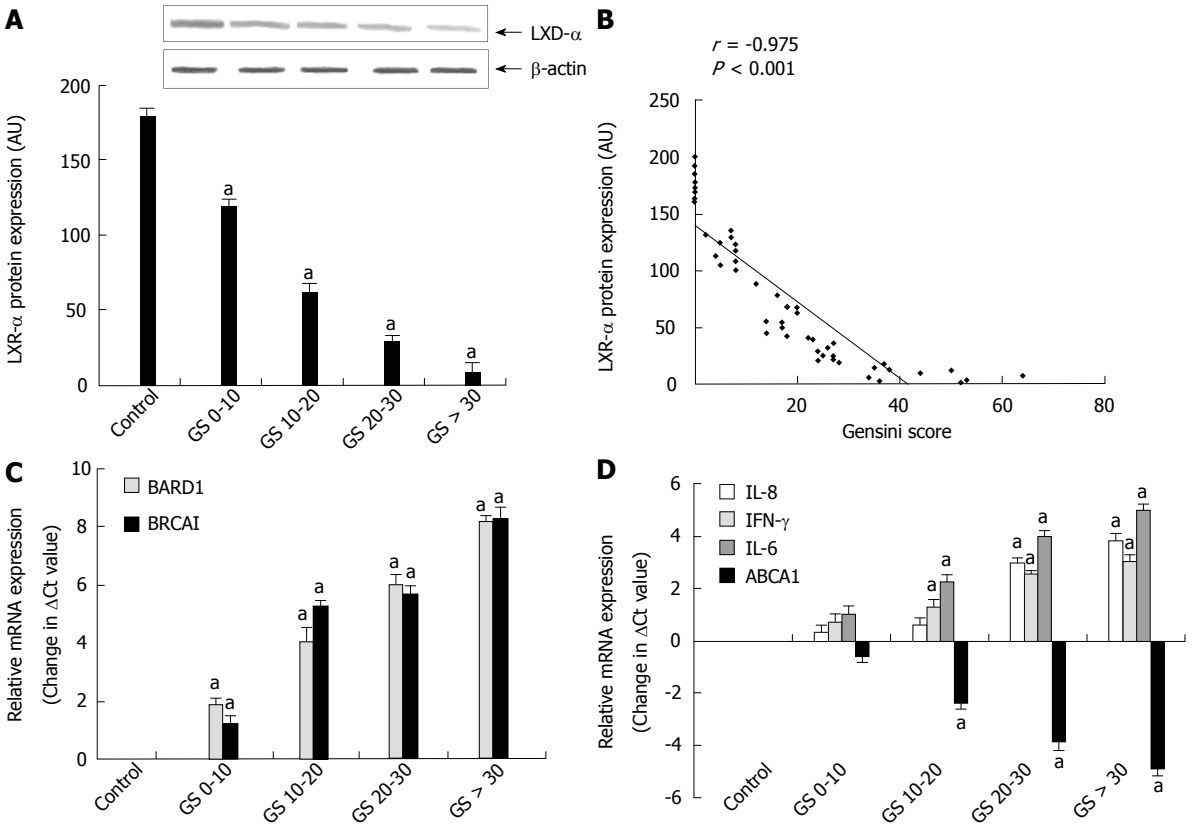



**Figure 1 Gene expression analysis of various genes with respect to gensini score.** A: Mean values of liver X receptor-α (LXR-α) protein levels in peripheral blood mononuclear cells (PBMCs) isolated from coronary heart disease (CHD) subjects and healthy controls with respect to increasing gensini score (reflecting the severity of coronary occlusion). Each bar represents mean ± SD for 10 different individuals in each group. The means were compared with one way ANOVA and $^{a}P < 0.05$ *vs* control group; B: Statistical correlation between translational expression of mutant LXR-α and gensini score within PBMCs. Values of r show Spearman rank correlation coefficient and $P < 0.01$ was considered statistically significant; C, D: Mean values of [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain 1] (BARD1) and BRCA1 (C) and interleukin (IL)-8, interferon (IFN)-γ, IL-6, and ATP-binding cassette A1 (ABCA1) (D) mRNA expression (change in ΔCt values) in PBMCs derived from CHD patients and healthy controls with respect to increasing Gensini Score. Each bar represents mean ± SD for 10 different individuals in each group. The means were compared with one way ANOVA and $^{a}P < 0.05$ *vs* control group.

complex in the degradation of mutant LXR-α (Figure 2C). Despite the increased expression of LXR-α protein upon cisplatin exposure, due to the absence of any ligand to activate mutant LXR-α, there was no effect on the expression of ABCA1 in both normal and patient cells (Figure 2D). BARD1/BRCA1 inhibition also causes a non-significant increase in LXR-α expression in normal cells where wild type ligand free LXR-α might escape degradation (Figure 2C).

## DISCUSSION

An alarming increase in CHD cases all over the world warrants molecular micro-dissection of pathways involved in the initiation and progression of CHD. LXR-α has been widely recognized as a master gene that plays a crucial role in cholesterol homeostasis, lipid peroxidation and inflammation responsible for the initiation of CHD and its clinical implications[1-7]. Apart from the observed protective effects of LXR-α agonists in the various cellular and animal model systems[8-14], the importance of LXR-α in pathogenesis of CHD is further highlighted by the studies from our laboratory which showed that both statins (drug of choice for CHD) and vitamin C have an inherent capacity to upregulate LXR-α expression[15] and also that human CHD subjects (with or without hyperlipidemia) have conspicuously higher blood cellular LXR-α mRNA expression as compared to their normal healthy counterparts[16]. However, synthetic agonists for the LXR-α activation designed as therapeutic agents for the regression of coronary atherosclerosis did not meet the expected success. This anomaly got further compounded by the observation in CHD patients who exhibited increasing transcriptional expression of LXR-α, within their PBMCs, with corresponding increase in severity of coronary occlusion[17]. This paradox was partly resolved by our earlier studies which showed that ligand binding domain of LXR-α protein in CHD subjects harbors a unique genetic aberration (involving Asp324, Pro327 and Arg328) which prevents its physiological ligands from binding and activating the LXR-α protein, thus rendering it non-functional[17]. Further, this mutant *LXR-α* gene product was shown to acquire by default affinity for Vitamin $D_3$ which can restore the function of mutated LXR-α protein to some extent[23].

The present study is based on the fact that ligand free LXR-α gets degraded by BARD1/BRCA1 heterodimer[18]. Since mutant LXR-α in CHD subjects is also unable to bind its natural physiological ligands, we at-

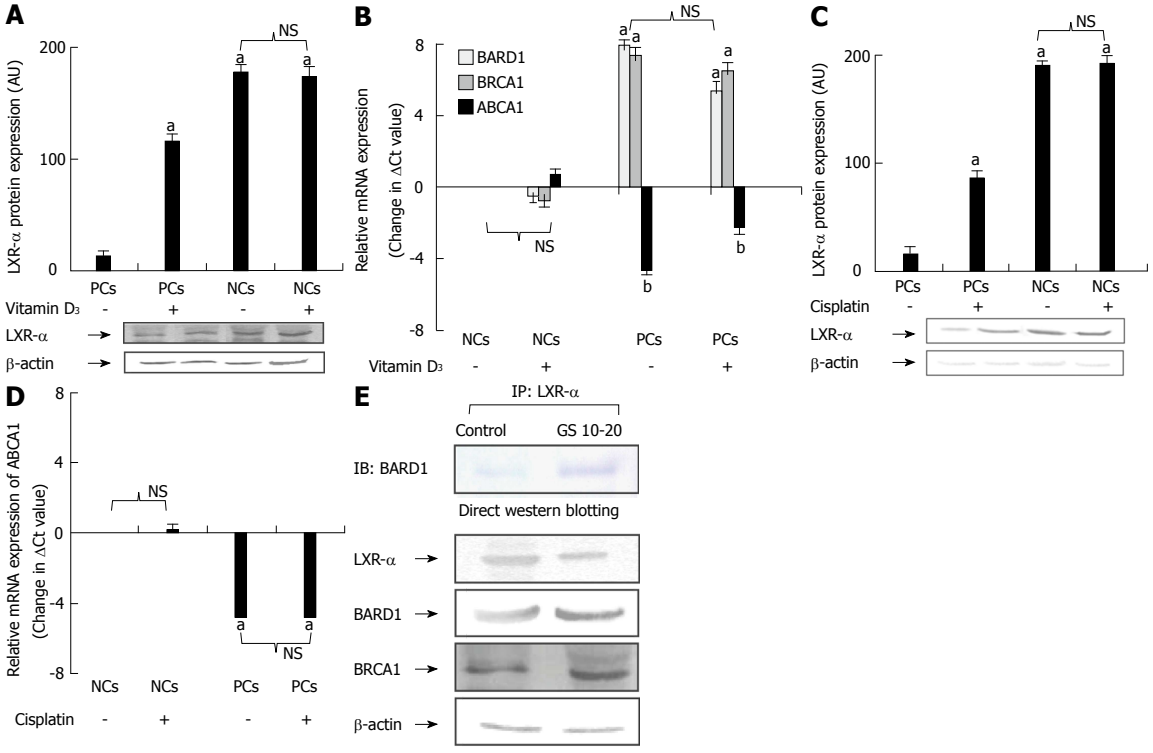



**Figure 2 Expression analysis of various genes upon exposure to Vitamin $D_3$ and Cisplatin in peripheral blood mononuclear cells of coronary heart diseasesubjects and their healthy counterparts.** A: Protein expression of liver X receptor-α (LXR-α) within peripheral blood mononuclear cells (PBMCs), isolated from coronary heart disease (CHD) subjects (GS > 30) as well as normal healthy controls, exposed to culture medium enriched with and without Vitamin $D_3$ (1 μmol/L); B: Relative mRNA expression (change in ΔCt values) of [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1] (BARD1), BRCA1 and ATP-binding cassette A1 (ABCA1) upon Vitamin $D_3$ exposure in normal and patient cells; C: Protein expression of LXR-α within PBMCs, isolated from CHD subjects (GS > 30) as well as normal healthy controls, exposed to culture medium enriched with and without Cisplatin (30 μmol/L); D: Relative mRNA expression (change in ΔCt values) of ABCA1 upon Cisplatin exposure in normal and patient cells. Each bar represents mean ± SD for the combined results of three independent experiments from different individuals in triplicate. The means were compared with one way ANOVA and $^aP < 0.05$ *vs* control group (A-D); E: Total cell lysates from PBMCs derived from CHD subjects and normal healthy individuals were immunoprecipitated with anti LXR-α antibody and immunoblotted with anti-BARD1 antibody. The direct western blotting shows the expression of LXR-α, BARD1, BRCA1 and β-actin (Sigma Aldrich) in PBMCs of CHD subjects and normal healthy controls. The experiments were repeated three times from different individuals and representative results are shown. IB: Immunoblotting; IP: Immunoprecipitation; NC: Normal cells; NS: Non-significant; PC: Patient cells.

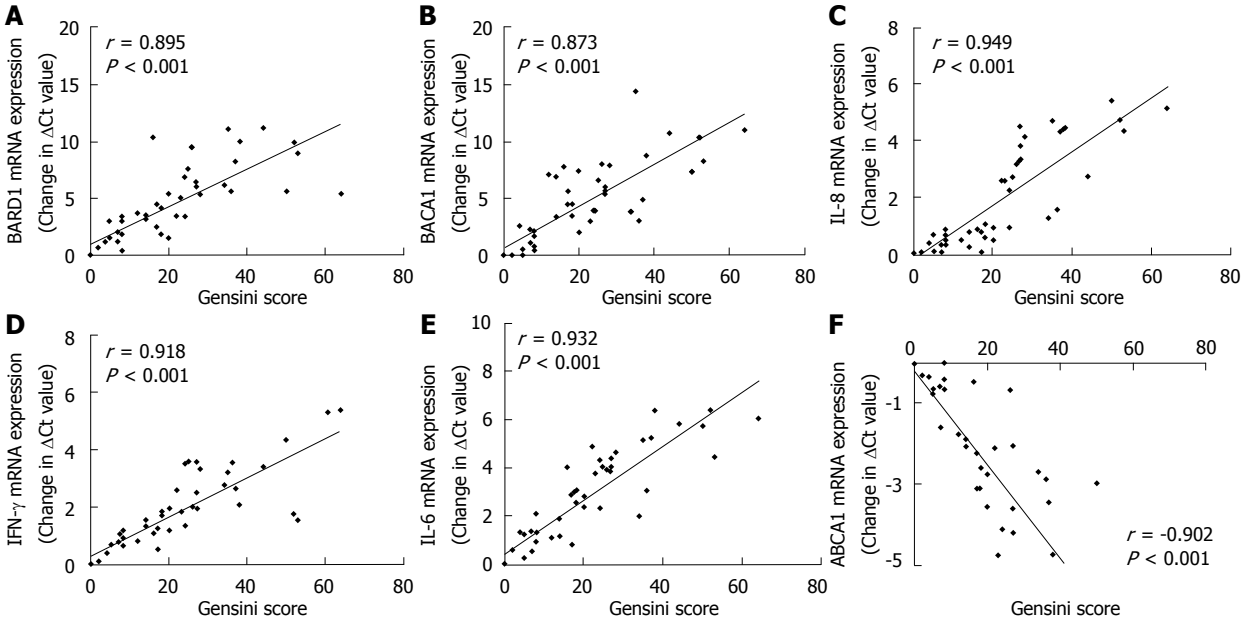



**Figure 3 Correlation of mRNA expression of various genes with the gensini score.** Statistical correlation between transcriptional expression of [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain1] (BARD1) (A), BRCA1 (B), interleukin (IL)-8 (C), interferon (IFN)-γ (D), IL-6 (E) and ATP-binding cassette A1 (ABCA1) (F) and gensini score within peripheral blood mononuclear cells derived from CHD subjects and normal healthy controls. Values of r show Spearman rank correlation coefficient.

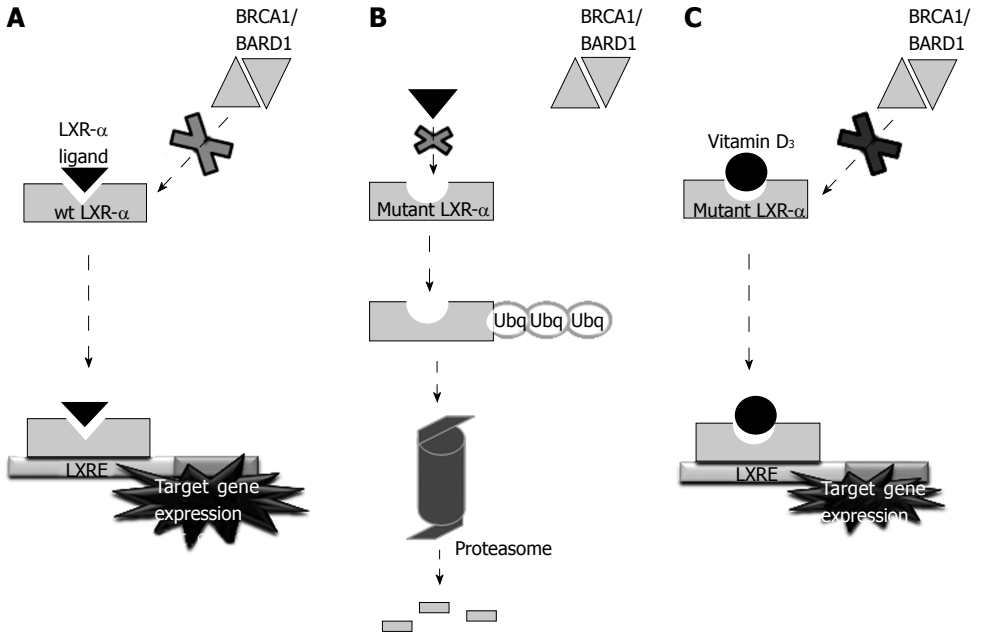



**Figure 4 Schematic diagram representing the mechanism of action of liver X receptor-α.** A: Wild type functional liver X receptor-α (LXR-α) in healthy controls; B: Mutant non-functional LXR-α in coronary heart disease patients; C: Mutated but functional (to some extent) LXR-α when rescued by Vitamin $D_3$. BRCA1: Breast and ovarian cancer susceptibility 1; BARD1: BRCA1-associated RING domain 1; LXRE: liver X receptor.

tempted to explore whether this heterodimeric complex plays any role in the decrease of LXR-α protein levels in such subjects. We performed certain preliminary experiments which showed strong correlation in the degradation of LXR-α and corresponding increase of BARD1/BRCA1 levels along with increasing disease severity (Figures 1A-C, 3A and B). This degradation of LXR-α protein also explains the increasing expression of inflammatory cytokines IFN-γ, IL-6 and IL-8 and decreasing expression of ABCA1 (responsible for cholesterol efflux particularly from macrophages) with increasing coronary occlusion (Figures 1D and 3C-F), which would ultimately result in increased vascular inflammation and foam cell formation. Thus, though the expression of LXR-α increases with the severity of the disease at the transcriptional level[17] (data not shown for subjects employed in present study), there is absence of functional LXR-α protein in CHD subjects (Figure 1A and B). To confirm the interaction between the two proteins, immunoprecipitation studies were performed which showed a strong association between mutant LXR-α and BARD1 in CHD subjects as compared to the normal healthy counterparts. A weak interaction observed between the two proteins in the healthy subjects could be explained by the presence of any ligand free wild type LXR-α which could also be bound and subsequently ubiquitinated by BARD1 (Figure 2E). To further precipitate the role of BARD1/BRCA1 in degradation of LXR-α *via* ubiquitination in CHD subjects, patient PBMCs were exposed to cisplatin (inhibitor of E3 ubiquitin ligase activity of BARD1/BRCA1[24]) *in vitro*. Though we observed an appreciable increase in LXR-α protein levels in PBMCs derived from CHD subjects upon cisplatin exposure, but it was still lower as compared to that in normal cells (Figure 2C). This may be explained by the fact that we used 30 μmol/L concentration of cisplatin as compared to 60 μmol/L used by Atipairin *et al*[25] (which reduced the E3 ubiquitin ligase activity of the BARD1/BRCA1 complex by half) which would have been toxic to the cells as in our case. Further since there is no ligand to modulate LXR-α transcriptional activity, the expression of ABCA1 is not affected upon cisplatin treatment (Figure 2D). In the previous study we have shown that Vitamin $D_3$ has an inherent capacity to activate mutant LXR-α in a dose dependent fashion[23]. Hence, it becomes imperative to examine whether or not the Vitamin $D_3$ bound to mutant LXR-α inhibits its degradation by BARD1/BRCA1. The results clearly showed that mutant LXR-α bound to Vitamin $D_3$ is rescued from degradation and not only brings the protein level of LXR-α close to that of normal healthy control (Figure 2A) but also activates it to some extent as can be seen by the increased expression of ABCA1 in patient cells exposed to Vitamin $D_3$ (Figure 2B). Further, Vitamin $D_3$ does not affect BARD1/BRCA1 expression (Figure 2B). But, Vitamin $D_3$ levels in the serum of CHD patients are significantly low[23,25-27] as can also be seen by the low serum Vitamin $D_3$ levels of the CHD subjects employed in the present study in comparison to their healthy counterparts (Table 1). The fact, that statins can upregulate Vitamin $D_3$ levels might add to their pleiotropic beneficial effects[23,28,29].

In conclusion, our findings provide evidence that mutant LXR-α in CHD patients is degraded by BARD1/BRCA1 E3 ubiquitin ligase complex and since Vitamin $D_3$ can rescue and simultaneously activate this mutant LXR-α (Figure 4), dietary supplementation of Vitamin

$D_3$ in such subjects may be exploited therapeutically. Further, *LXR-α* gene mutation and the extent of LXR-α protein degradation may be exploited as potential non-invasive markers for early diagnosis and prognosis, as well as for predicting the susceptibility of an individual to develop the disease in future. However, population studies are warranted to substantiate these propositions.

## COMMENTS

### Background

Atherosclerosis and its clinical manifestations, such as myocardial infarction or stroke, are the leading causes of morbidity and mortality in the modern world. Lipid peroxidation and inflammation are the two hallmarks of atherosclerotic lesion development. Though lipid-lowering agents like statins are the drug of choice, they do not provide complete protection, thus necessitating an in-depth dissection of other critical molecular pathways that could alter the disease course.

### Research frontiers

Liver X receptor-α (LXR-α) is a key athero-protective molecule regulating cholesterol homeostasis as well as inflammation. Various *in vitro* and *in vivo* studies in mice models have demonstrated the protective role of LXR-α. The use of various ligands for activating LXR-α, while avoiding its side effects, is a research hotspot in this area. Also, the association of Vitamin $D_3$ deficiency with atherosclerosis progression and its dietary supplementation to prevent or treat atherosclerosis is another major area of research in relation to atherosclerosis.

### Innovations and breakthroughs

Previous studies in the authors' laboratory have demonstrated that the ligand binding domain of LXR-α is mutated in coronary heart disease (CHD) subjects, thus rendering it incapable of binding and getting activated by its natural physiological ligands. Recent studies have established that ligand-free LXR-α gets ubiquitinated and subsequently targeted for proteasomal degradation, by [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain1 (BARD1)]/BRCA1 E3 ubiquitin ligase complex. Accordingly, in the present study we explored the role of BARD1/BRCA1 complex in governing the stability of mutant LXR-α in these subjects. Also a specific inhibitor of BARD1/BRCA1 complex, Cisplatin, was used to warranty the claimed results. They also investigated the role of Vitamin $D_3$, a natural ligand of mutant LXR-α, in preventing its degradation by ubiquitination.

### Applications

The authors' findings suggest that dietary supplementation of Vitamin $D_3$ in CHD subjects may be exploited therapeutically. Further, *LXR-α* gene mutation and the extent of LXR-α protein degradation may be exploited as potential non-invasive markers for early diagnosis and prognosis, as well as for predicting the susceptibility of an individual to develop the disease in future.

### Terminology

Atherosclerosis is a chronic inflammatory response to the accumulation of macrophages and white blood cells in the walls of arteries, promoted by low-density lipoproteins without adequate removal of fats and cholesterol from the macrophages. LXR-α is a ligand dependent transcription factor belonging to nuclear receptor super-family. It forms heterodimer with the LXR and binds to the regulatory region of target genes, modulating their expression upon ligand binding. BARD1/BRAC1 forms a functional heterodimer having an ubiquitin ligase activity that targets specific proteins for proteasomal degradation.

### Peer review

The authors explored the role of BARD1/BRCA1 heterodimer in governing the stability of mutant LXR-α protein in CHD subjects. It is an excellent study.

## REFERENCES


1 **Zelcer N**, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest* 2006; **116**: 607-614 [PMID: 16511593 DOI: 10.1172/JCI27883]

2 **Blaschke F**, Leppanen O, Takata Y, Caglayan E, Liu J, Fishbein MC, Kappert K, Nakayama KI, Collins AR, Fleck E, Hsueh WA, Law RE, Bruemmer D. Liver X receptor agonists suppress vascular smooth muscle cell proliferation and inhibit neointima formation in balloon-injured rat carotid arteries. *Circ Res* 2004; **95**: e110-e123 [PMID: 15539633 DOI: 10.1161/01.RES.0000150368.56660.4f]

3 **Joseph SB**, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med* 2003; **9**: 213-219 [PMID: 12524534 DOI: 10.1038/nm820]

4 **Michael DR**, Ashlin TG, Buckley ML, Ramji DP. Liver X receptors, atherosclerosis and inflammation. *Curr Atheroscler Rep* 2012; **14**: 284-293 [PMID: 22419222 DOI: 10.1007/s11883-012-0239-y]

5 **Kidani Y**, Bensinger SJ. Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. *Immunol Rev* 2012; **249**: 72-83 [PMID: 22889216 DOI: 10.1111/j.1600-065X.2012.01153.x]

6 **Repa JJ**, Mangelsdorf DJ. The liver X receptor gene team: potential new players in atherosclerosis. *Nat Med* 2002; **8**: 1243-1248 [PMID: 12411951 DOI: 10.1038/nm1102-1243]

7 **Tontonoz P**, Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol* 2003; **17**: 985-993 [PMID: 12690094 DOI: 10.1210/me.2003-0061]

8 **Calkin AC**, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010; **30**: 1513-1518 [PMID: 20631351 DOI: 10.1161/ATVBAHA.109.191197]

9 **Joseph SB**, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, Tran J, Tippin TK, Wang X, Lusis AJ, Hsueh WA, Law RE, Collins JL, Willson TM, Tontonoz P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci USA* 2002; **99**: 7604-7609 [PMID: 12032330 DOI: 10.1073/pnas.112059299]

10 **Levin N**, Bischoff ED, Daige CL, Thomas D, Vu CT, Heyman RA, Tangirala RK, Schulman IG. Macrophage liver X receptor is required for antiatherogenic activity of LXR agonists. *Arterioscler Thromb Vasc Biol* 2005; **25**: 135-142 [PMID: 15539622]

11 **Tangirala RK**, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, Daige CL, Thomas D, Heyman RA, Mangelsdorf DJ, Wang X, Lusis AJ, Tontonoz P, Schulman IG. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci USA* 2002; **99**: 11896-11901 [PMID: 12193651 DOI: 10.1073/pnas.182199799]

12 **Terasaka N**, Hiroshima A, Koieyama T, Ubukata N, Morikawa Y, Nakai D, Inaba T. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett* 2003; **536**: 6-11 [PMID: 12586329 DOI: 10.1016/S0014-5793(02)03578-0]

13 **Laemmli UK**. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685 [PMID: 5432063]

14 **Verschuren L**, de Vries-van der Weij J, Zadelaar S, Kleemann R, Kooistra T. LXR agonist suppresses atherosclerotic lesion growth and promotes lesion regression in apoE*3Leiden mice: time course and mechanisms. *J Lipid Res* 2009; **50**: 301-311 [PMID: 18757914 DOI: 10.1194/jlr.M800374-JLR200]

15 **Verschuren L**, de Vries-van der Weij J, Zadelaar S, Kleemann R, Kooistra T. LXR agonist suppresses atherosclerotic lesion growth and promotes lesion regression in apoE*3Leiden mice: time course and mechanisms. *J Lipid Res* 2009; **50**: 301-311 [PMID: 18757914 DOI: 10.1194/jlr.M800374-JLR200]

16 **Kaul D**, Baba MI. Genomic effect of vitamin 'C' and statins within human mononuclear cells involved in atherogenic process. *Eur J Clin Nutr* 2005; **59**: 978-981 [PMID: 15970944 DOI: 10.1038/sj.ejcn.1602203]

17 **Baba MI**, Kaul D, Grover A. Importance of blood cellular genomic profile in coronary heart disease. *J Biomed Sci* 2006; **13**: 17-26 [PMID: 16252156 DOI: 10.1007/s11373-005-9041-y]

18 **Dave VP**, Kaul D, Sharma Y, Bhattacharya R. Functional

genomics of blood cellular LXR-alpha gene in human coronary heart disease. *J Mol Cell Cardiol* 2009; **46**: 536-544 [PMID: 19211025 DOI: 10.1016/j.yjmcc.2008.12.020]
19 **Kim KH**, Yoon JM, Choi AH, Kim WS, Lee GY, Kim JB. Liver X receptor ligands suppress ubiquitination and degradation of LXRalpha by displacing BARD1/BRCA1. *Mol Endocrinol* 2009; **23**: 466-474 [PMID: 19164445 DOI: 10.1210/me.2008-0295]
20 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Postgrad Med* 2002; **48**: 206-208 [PMID: 12432198]
21 **Gensini GG**. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; **51**: 606 [PMID: 6823874 DOI: 10.1016/S0002-9149(83)80105-2]
22 **Böyum A**. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl* 1968; **97**: 77-89 [PMID: 4179068]
23 **Chomczynski P**, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159 [PMID: 2440339 DOI: 10.1016/0003-2697(87)90021-2]
24 **Dave VP**, Kaul D, Sharma YP, Bhattacharya R, Dhawan V. Mutated LXR-A Gene within Blood Mononuclear Cells of CHD Patients: Significance of Serum Factors. *J Clinic Experiment Cardiol* 2011; **2**: 1000125 [DOI: 10.4172/2155-9880.1000125]
25 **Atipairin A**, Canyuk B, Ratanaphan A. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by the platinum-based anticancer drugs. *Breast Cancer Res Treat* 2011; **126**: 203-209 [PMID: 20878461 DOI: 10.1007/s10549-010-1182-7]
26 **Dobnig H**, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008; **168**: 1340-1349 [PMID: 18574092 DOI: 10.1001/archinte.168.12.1340]
27 **Giovannucci E**, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008; **168**: 1174-1180 [PMID: 18541825 DOI: 10.1001/archinte.168.11.1174]
28 **Pilz S**, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, März W. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008; **39**: 2611-2613 [PMID: 18635847 DOI: 10.1161/STROKEAHA.107.513655]
29 **Pérez-Castrillón JL**, Vega G, Abad L, Sanz A, Chaves J, Hernandez G, Dueñas A. Effects of Atorvastatin on vitamin D levels in patients with acute ischemic heart disease. *Am J Cardiol* 2007; **99**: 903-905 [PMID: 17398180 DOI: 10.1016/j.amjcard.2006.11.036]
30 **Yavuz B**, Ertugrul DT, Cil H, Ata N, Akin KO, Yalcin AA, Kucukazman M, Dal K, Hokkaomeroglu MS, Yavuz BB, Tutal E. Increased levels of 25 hydroxyvitamin D and 1,25-dihydroxyvitamin D after rosuvastatin treatment: a novel pleiotropic effect of statins? *Cardiovasc Drugs Ther* 2009; **23**: 295-299 [PMID: 19543962 DOI: 10.1007/s10557-009-6181-8]

**P- Reviewer** Das UN **S- Editor** Zhai HH
**L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.313

*World J Cardiol* 2013 August 26; 5(8): 313-316
ISSN 1949-8462 (online)


CASE REPORT

# Longitudinal stent compression of everolimus-eluting stent: A report of 2 cases

Rajesh Vijayvergiya, Alok Kumar, Smit Shrivastava, Naveen K Kamana

Rajesh Vijayvergiya, Alok Kumar, Smit Shrivastava, Naveen K Kamana, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Author contributions: All authors were actively involved in management of the index cases.

Correspondence to: Dr. Rajesh Vijayvergiya, MD, DM, FSCAI, FISES, FACC, Associate Professor, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. rajeshvijay999@hotmail.com

Telephone: +91-172-2756512 Fax: +91-172-2744401

Received: March 19, 2013 Revised: May 1, 2013

Accepted: July 17, 2013

Published online: August 26, 2013

## Abstract

Second generation drug eluting stents (DES) have shown better safety and efficacy in comparison to first generation DES, because of thinner struts, nondurable polymers and coating with better anti-proliferative drugs. The newer DES with cobalt alloy base have demonstrated a greater trackability, deliverability, conformability, flexibility and radio-opacity. However, these thin strut stents have a downside of poor longitudinal axial strength, and therefore get easily deformed/compressed at their end with a slight trauma during exchange of various catheters. We hereby report two cases of "longitudinal stent compression (LSC)" of everolimus-eluting stent, which happened during percutaneous coronary intervention of right coronary artery. Both the cases were successfully managed with non-compliant balloon dilatation. Various reasons for LSC and its management are discussed in the article.



**Key words:** Complication; Everolimus-eluting stent; Longitudinal stent compression; Percutaneous coronary intervention; Stent structure; Stent deformation

**Core tip:** The newer second generation drug eluting stent (DES) have shown a greater safety and efficacy compared to first generation DES, because of thinner struts, nondurable polymers and coating with better anti-proliferative drugs. Though their performance is excellent for various type of coronary lesions, one downside is that they are susceptible for compression/deformation because of poor longitudinal axial strength. We came across longitudinal stent compression (LSC) of everolimus-eluting stent in two cases, which was successfully managed by balloon dilatation. Various reasons for LSC and its management are discussed in the article.

Vijayvergiya R, Kumar A, Shrivastava S, Kamana NK. Longitudinal stent compression of everolimus-eluting stent: A report of 2 cases. *World J Cardiol* 2013; 5(8): 313-316 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/313.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.313

## INTRODUCTION

Second generation drug eluting stents (DES) have shown better safety and efficacy in comparison to first generation DES, because of thinner struts, nondurable polymers and coating with better anti-proliferative drugs[1,2]. A change in stent platform from stainless steel to cobalt alloy and a change in stent design have improved the performance of newer DES in terms of trackability, deliverability, conformability, flexibility and radio-opacity. However, these thin-strut stent have a downside of poor longitudinal axial strength, resulting into a newly described observation of "longitudinal stent compression (LSC)"[3]. We hereby report two cases of LSC with everolimus-eluting PROMUS Element stent.

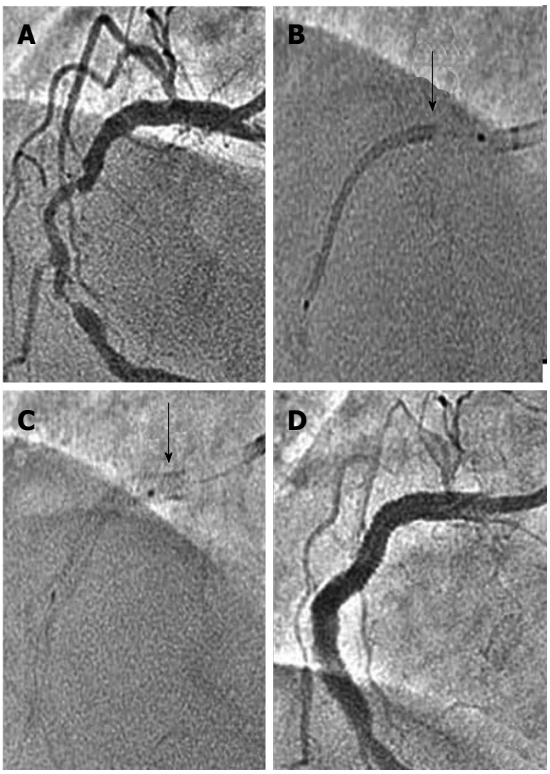



**Figure 1 Percutaneous intervention of mid right coronary artery in case 1.** A: 90% eccentric, calcified, type C lesion of mid right coronary artery (RCA); B: Longitudinal compression of un-inflated stent in proximal RCA as marked by a black arrow; C: Post stent deployment, the longitudinally compressed proximal part of stent as marked by a black arrow; D: Final result showing thrombolysis in myocardial infarction-3 flow in RCA.

## CASE REPORT

### *Case 1*

A 62-year-old hypertensive male presented with acute anterior wall myocardial infarction (MI) in July 2012. He underwent primary angioplasty and stenting of mid left anterior descending (LAD) artery. Five days later, he had elective percutaneous coronary angioplasty (PCI) of right coronary artery (RCA). The dominant mid RCA showed a 90% type C, eccentric, calcified lesion (Figure 1A). The RCA was cannulated with JR 3.5, 6F guide catheter, lesion was crossed with 0.014 inch guide wire (Zinger-Support wire; Medtronic, Inc., Minneapolis, Minnesota), and dilated with 2.5 mm × 15 mm semi-compliant balloon (Sprinter balloon, Medtronic). Thereafter, a 3.5 mm × 38 mm PROMUS Element™ stent (Boston Scientific, Natick, MA, United States) was taken for deployment, but it could not be pushed across the calcified mid RCA lesion. During forceful manipulations to push it, the distal end of stent got stuck-up at mid RCA. Thereafter, an attempt to pull it back into the guide catheter resulted into longitudinal compression of proximal end of the stent (Figure 1B and C). The stent got dislodged from the stent balloon at its proximal position (Figure 1B). At this point, the stent was deployed at same position without any further manipulation. Following stent deployment, the stented segment was post-dilated with 3.5 mm × 15 mm non-compliant (NC) balloon (Sprinter balloon, Medtronic). The residual mid RCA lesion, distal to the deployed stent was dilated with 3.0 mm × 15 mm NC balloon, and a 3.5 mm × 25 mm bare-metal stent (Skylor stent, Medtronic-Invatec, Roncadelle, Italy) was deployed, overlapping the proximal stent. The whole stented segment was post-dilated with 3.5 mm × 15 mm NC balloon at 18 atmospheres. RCA had a thrombolysis in myocardial infarction (TIMI)-3 flow at the end of procedure (Figure 1D). There was no hemodynamic instability during the intervention. He remained asymptomatic during follow-up and a check angiogram at 9-mo showed patent RCA and LAD stents.

### *Case 2*

A 65-years-old male presented with 15-d old anterior wall MI in July 2012. He was in gross congestive heart failure, which improved with diuretic therapy. Echocardiography revealed akinetic anterior wall, no mitral regurgitation and ejection fraction of 0.30. Coronary angiogram revealed 100% occluded proximal LAD with thrombus, and a 90% eccentric, calcified, type C lesion at proximal RCA (Figure 2A). He was subjected for PCI to LAD. The left coronary artery was cannulated with JL 3.5, 6 F guide catheter and proximal LAD lesion was crossed with 0.014 inch guide wire (Zinger-Support wire, Medtronic). The lesion was dilated with 2.5 mm × 15 mm balloon and thrombus aspiration with 6F Export aspiration catheter (Medtronic) was performed. There was TIMI-0 flow despite repeated thrombus aspiration and intra-coronary bolus of abciximab. He was put on abciximab infusion and shifted back to coronary care unit. Later, a check angiogram showed occluded proximal LAD. This time no further intervention was performed to LAD considering it as a non-viable territory; and was taken up for PCI to RCA. The RCA was cannulated with JR 3.5, 6 F guide catheter and proximal RCA lesion was crossed with 0.014 inch guide wire (Zinger-Support wire, Medtronic). The lesion was dilated with 2.5 mm × 15 mm semi-compliant balloon (Sprinter, Medtronic). Thereafter, a 2.75 mm × 38 mm PROMUS Element™ stent (Boston Scientific) was taken for deployment, but it could not be pushed across calcified proximal RCA lesion. During forceful manipulations to push it, the distal end of stent got stuck-up at proximal lesion site. Thereafter, an attempt to pull it back into the guide catheter resulted into longitudinal compression of proximal end of the stent (Figure 2B and C). The stent got dislodged from the stent balloon at its proximal position (Figure 2C). The stent was deployed at same position without any further manipulation. Post stent deployment, the stented segment was post-dilated with 2.75 mm × 15 mm NC balloon (Sprinter, Medtronic). The residual RCA lesion, distal to the deployed stent was dilated with 2.75 mm × 15 mm NC balloon and a 2.75 mm × 16 mm PROMUS Element™ stent (Boston Scientific) was deployed, overlapping the proximal stent. The whole stented segment was post-dilated with 2.75 mm × 15 mm NC balloon at 18 atmospheres. He remained he-

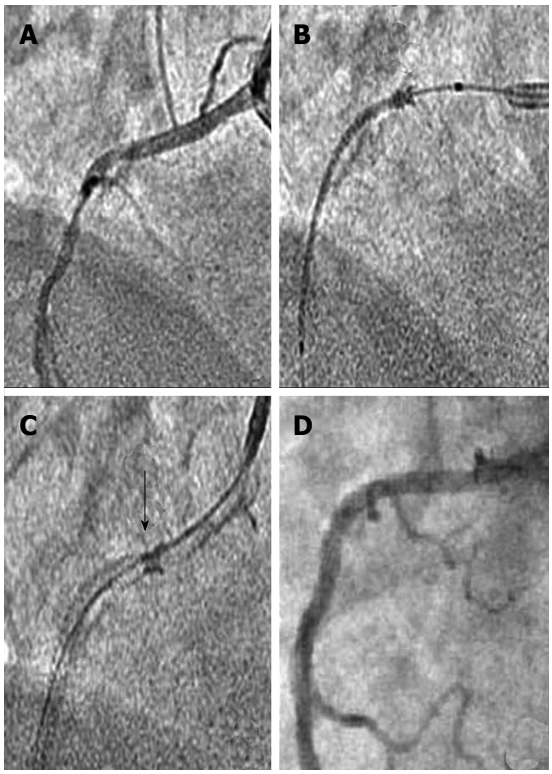



**Figure 2 Percutaneous intervention of mid right coronary artery in case 2.** A: 90% eccentric, calcified, type C lesion of proximal right coronary artery (RCA); B: Longitudinal compression of un-inflated stent in proximal RCA as marked by a black arrow; C: Post stent deployment, the longitudinally compressed proximal part of stent as marked by a black arrow; D: Final result showing thrombolysis in myocardial infarction-3 flow in RCA.

modynamically stable during PCI and had a TIMI-3 flow in RCA (Figure 2D). Twenty-four hours later, he had an episode of massive hematemesis followed by hypotension, which was appropriately managed. There was no chest pain and no ST-segment elevation in inferior electrocardiogram leads, which rule out a possibility of acute stent-thrombosis. Later in the course, he had recurrent ventricular tachycardia followed by asystole, from which he could not be revived and expired.

## DISCUSSION

Longitudinal stent compression has been described by various authors in newer generation cobalt alloy stents. It is commonly reported with PROMUS Element stent, though isolated report of other stents such as Taxus Liberte (Boston Scientific Co., Natick, MA, United States), Biomatrix (Biosensors Interventional Technologies, Singapore), Endeavor (Medtronic Inc., Minneapolis, Minnesota) and Xience (Abbott Vascular, Santa Clara, CA, United States) is also available[4,5]. Our incidence of 0.8% LSC (2 out of 250 deployed PROMUS Element stents in 6 mo, from July 2012-December 2012) is similar to the reported incidence of 0.6%-0.8% by other authors[4-6]. A bench testing for longitudinal strength of various DES as studied by Ormiston *et al*[7] and Prabhu *et al*[8], have demonstrated that a 2-link offset peak-to-peak stent design of PROMUS Element has the lowest resistance for longitudinal compression. A relatively better radio-opacity of PROMUS Element is another reason for frequent recognition of LSC during fluoroscopy[9]. The incidence can be much higher with various stents, if a routine intravascular ultrasound (IVUS) or optical coherence tomography (OCT) is performed[3,10]. Various reasons for LSC can be guide catheter induced deformation of a stent at osteal or proximal lesions and a compression by un-inflated balloon or IVUS catheter[3,4]. In both of our cases, the stent got stuck up in calcified RCA lesion, and during attempted withdrawal of stent in guide catheter, it got longitudinally compressed at proximal end by catheter tip. A guide catheter induced compression was the reason for stent deformation in all the three reported cases by Hanratty *et al*[3]. We personally feel that a better plaque modification in a calcified/hard lesion is mandatory prior to stent deployment to avoid such complication. Proper handling and a resistance free passage of various catheters across the stented segment can prevent LSC. The treatment of LSC includes dilatation of deformed segment with adequate size non-compliant balloon and if required another stent for favorable end results[4]. In both the cases, we had favorable outcome after non-compliant balloon dilatation and without putting an additional stent at deformed site. Though, fluoroscopy has a limited value in comparison to IVUS or OCT for diagnosis of malapposition of deformed stent segment, we did not perform it in both the cases. Willims *et al*[4] have reported a case of stent thrombosis following LSC at mid LAD. The reasons for death of 2nd index case were multi-factorial including upper gastro-intestinal bleed, hypotension secondary to blood loss, ischemic heart failure, and recurrent ventricular tachycardia; however a possible acute stent thrombosis of RCA could not be ruled out.

In conclusion, LSC is a rare phenomenon, which is observed with most of newer thin-strut DES. PROMUS Element having a 2-link offset peak-to-peak stent design is more prone to longitudinal compression in comparison to other stents. A meticulous PCI technique with proper handling of various catheters across the ostio-proximal lesions and stented segment is important to avoid such complication. A timely recognition with available imaging modality such as fluoroscopy, IVUS or OCT and an appropriate treatment is essential to avoid unfavorable clinical outcome.

## REFERENCES

1 **Kedhi E**, Gomes ME, Lagerqvist B, Smith JG, Omerovic E, James S, Harnek J, Olivecrona GK. Clinical impact of second-generation everolimus-eluting stent compared with first-generation drug-eluting stents in diabetes mellitus patients: insights from a nationwide coronary intervention register. *JACC Cardiovasc Interv* 2012; **5**: 1141-1149 [PMID: 23174638 DOI: 10.1016/j.jcin.2012.06.020]

2 **Planer D**, Smits PC, Kereiakes DJ, Kedhi E, Fahy M, Xu K, Serruys PW, Stone GW. Comparison of everolimus- and paclitaxel-eluting stents in patients with acute and stable coronary syndromes: pooled results from the SPIRIT (A

Clinical Evaluation of the XIENCE V Everolimus Eluting Coronary Stent System) and COMPARE (A Trial of Everolimus-Eluting Stents and Paclitaxel-Eluting Stents for Coronary Revascularization in Daily Practice) Trials. *JACC Cardiovasc Interv* 2011; **4**: 1104-1115 [PMID: 22017936 DOI: 10.1016/j.jcin.2011.06.018]

3 **Hanratty CG**, Walsh SJ. Longitudinal compression: a "new" complication with modern coronary stent platforms--time to think beyond deliverability? *EuroIntervention* 2011; **7**: 872-877 [PMID: 21970984 DOI: 10.4244/EIJV7I7A135]

4 **Williams PD**, Mamas MA, Morgan KP, El-Omar M, Clarke B, Bainbridge A, Fath-Ordoubadi F, Fraser DG. Longitudinal stent deformation: a retrospective analysis of frequency and mechanisms. *EuroIntervention* 2012; **8**: 267-274 [PMID: 22052084 DOI: 10.4244/EIJV8I2A41]

5 **Shannon J**, Latib A, Takagi K, Chieffo A, Figini F, Sacco F, Ferrarello S, Montorfano M, Colombo A. "Procedural trauma risks longitudinal shortening of the Promus Element™ stent platform". *Catheter Cardiovasc Interv* 2013; **81**: 810-817 [PMID: 22899552 DOI: 10.1002/ccd.24600]

6 **Leibundgut G**, Gick M, Toma A, Valina C, Löffelhardt N, Büttner HJ, Neumann FJ. Longitudinal compression of the platinum-chromium everolimus-eluting stent during coronary implantation: predisposing mechanical properties, incidence, and predictors in a large patient cohort. *Catheter Cardiovasc Interv* 2013; **81**: E206-E214 [PMID: 22581708 DOI: 10.1002/ccd.24472]

7 **Ormiston JA**, Webber B, Webster MW. Stent longitudinal integrity bench insights into a clinical problem. *JACC Cardiovasc Interv* 2011; **4**: 1310-1317 [PMID: 22136972 DOI: 10.1016/j.jcin.2011.11.002]

8 **Prabhu S**, Schikorr T, Mahmoud T, Jacobs J, Potgieter A, Simonton C. Engineering assessment of the longitudinal compression behaviour of contemporary coronary stents. *EuroIntervention* 2012; **8**: 275-281 [PMID: 22057097 DOI: 10.4244/EIJV8I2A42]

9 **Finet G**, Rioufol G. Coronary stent longitudinal deformation by compression: is this a new global stent failure, a specific failure of a particular stent design or simply an angiographic detection of an exceptional PCI complication? *EuroIntervention* 2012; **8**: 177-181 [PMID: 22057125 DOI: 10.4244/EIJV8I2A29]

10 **Bartorelli AL,** Andreini D, Pontone G, Trabattoni D, Ferrari C, Mushtaq S, Ormiston JA. Stent longitudinal distortion: strut separation (pseudo-fracture) and strut compression ("concertina" effect). *EuroIntervention* 2012; **8**: 290-291 [PMID: 22717930 DOI: 10.4244/EIJV8I2A44]

**P- Reviewers** Cheng XS, Prashanth P, Teng RJ
**S- Editor** Gou SX **L- Editor** A **E- Editor** Lu YJ

World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
www.wjgnet.com

*World J Cardiol* 2013 August 26; 5(8): I-V
ISSN 1949-8462 (online)


*INSTRUCTIONS TO AUTHORS*

## GENERAL INFORMATION

*World Journal of Cardiology* (*World J Cardiol*, *WJC*, online ISSN 1949-8462, DOI: 10.4330) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

### *Aim and scope*

*WJC* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of cardiology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJC*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

*WJC* is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial board members or peer reviewers, and is a world first-class publisher.

### *Columns*

The columns in the issues of *WJC* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in cardiology; (12) Brief Articles: To briefly report the novel and innovative findings in cardiology; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, e.g., the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJC*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of cardiology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

### *Name of journal*

*World Journal of Cardiology*

### *ISSN*

ISSN 1949-8462 (online)

### *Launch date*

December 31, 2009

### *Frequency*

Monthly

***Editorial office***
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
*World Journal of Cardiology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjc@wjgnet.com
http://www.wjgnet.com

***Publisher***
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

***Production center***
Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

***Representative office***
USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States

***Instructions to authors***
Full instructions are available online at http://www.wjgnet.com/1949-8462/g_info_20100316161927.htm.

***Indexed and Abstracted in***
PubMed Central, PubMed, Digital Object Identifier, and Directory of Open Access Journals.

## SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

***Biostatistical editing***
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

***Conflict-of-interest statement***
In the interests of transparency and to help reviewers assess any potential bias, *WJC* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

***Statement of informed consent***
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

***Statement of human and animal rights***
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

## SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and report-

ing of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

### *Online submissions*

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1949-8462/g_info_20100316161927.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjc@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

## MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

### *Title page*

**Title:** Title should be less than 12 words.

**Running title:** A short running title of less than 6 words should be provided.

**Authorship:** Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

**Institution:** Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

**Author contributions:** The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

**Supportive foundations:** The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

**Correspondence to:** Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

**Telephone and fax:** Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

**Peer reviewers:** All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

### *Abstract*

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, $6.92 \pm 3.86$ *vs* $3.61 \pm 1.67$, $P < 0.001$), and CONCLUSION (no more than 26 words).

### *Key words*

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

### *Core tip*

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

### *Text*

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

### *Illustrations*

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

### *Tables*

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a

second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

### *Notes in tables and illustrations*

Data that are not statistically significant should not be noted. $^{a}P <$ 0.05, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of $P$ values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of $P$ values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as $^{1}$F, $^{2}$F, $^{3}$F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

### *Acknowledgments*

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

## REFERENCES

### *Coding system*

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

### *PMID and DOI*

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

### *Style for journal references*

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

### *Style for book references*

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

### *Format*

**Journals**

*English journal article (list all authors and include the PMID where applicable)*

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

*Chinese journal article (list all authors and include the PMID where applicable)*

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

*In press*

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

*Organization as author*

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

*Both personal authors and an organization as author*

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

*No author given*

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

*Volume with supplement*

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

*Issue with no volume*

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

*No volume or issue*

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

**Books**

*Personal author(s)*

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

*Chapter in a book (list all authors)*

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

*Author(s) and editor(s)*

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

*Conference proceedings*

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

*Conference paper*

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

**Electronic journal** (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

**Patent** (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

***Statistical data***
Write as mean ± SD or mean ± SE.

***Statistical expression***
Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as $\chi^2$ (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

***Units***
Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; $CO_2$ volume fraction, 50 mL/L $CO_2$, not 5% $CO_2$; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23243641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1949-8462/g_info_20100312200347.htm.

***Abbreviations***
Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

***Italics***
Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

***Examples for paper writing***
All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

## RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system via the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

***Language evaluation***
The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

***Copyright assignment form***
Please download a Copyright assignment form from http://www.wjgnet.com/1949-8462/g_info_20100312200118.htm.

***Responses to reviewers***
Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1949-8462/g_info_20100312195923.htm.

***Proof of financial support***
For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

## STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

## PUBLICATION FEE

*WJC* is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. All invited articles are published free of charge.

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com