WORLD HEART JOURNAL

Volume 6, Issue 2, 2014

TABLE OF CONTENTS

nova publishers
New York

World Heart Journal

Topics to be covered in the *WHJ* include the following: Epidemiology and Prevention, Chronocardiology and Chronomics, Nutrition and Lifestyle in CVD, Clinical Cardiology, Cardiovascular Sciences (Molecular Cardiology: biochemistry and biology), Hypertension, Coronary artery disease, Pharmacotherapy, Electrophysiology, Echocardiography, Nuclear Cardiology, Pediatric Cardiology, Geriatric Cardiology, CVD in women, Cardiac Rehabilitation and Prehabilitation, as well as Interventional Cardiology and Cardiac surgery.

World Heart Journal
is published quarterly by

Nova Science Publishers, Inc.
400 Oser Avenue, Suite 1600
Hauppauge, New York, 11788-3619, U.S.A.
Telephone: (631) 231-7269
Fax: (631) 231-8175
E-mail: nova.main@novapublishers.com
Web: www.novapublishers.com

ISSN: 1556-4002

Subscription Price per Volume
Print: $500 Electronic: $500 Combined Print + Electronic: $750

Additional color graphics might be available in the e-version of this journal.

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Editorial - Antiplatelet Therapy in Heart Failure

Jan Fedacko[1], Ram B. Singh[2], Teodora Handjiev Darlenska[*3], Krasimira Hristova[4], Daniel Pella[1], and Hilton Chaves[5]

[1]Faculty of Medicine, PJ Safaric University, Kosice, Slovakia
[2]Halberg Hospital and Research Institute, Moradabad, India
[3]Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University, Sofia, Bulgaria
[4]National Heart Hospital, Sofia, Bulgaria, PJ Safaric University, Kosice, Slovakia
[5]Federal University of Pernambuco, Recefe, Brazil

The prevalence and incidence of heart failure (HF), based on disease-specific estimates may be conservatively attributed mainly due to coronary artery disease (CAD), hypertension, obesity, diabetes and rheumatic heart disease. The prevalence rate range from 1.3 to 4.6 million, with an annual incidence of 491 600-1.8 million [1,2]. However, the causes of HF in the Western World are CAD, cardiomyopathy, hypertension, obesity and diabetes mellitus [1,2]. Although, no data are available from developing and newly industrialized countries, regarding the exact prevalence and incidence of HF, it is possible that higher propensity for CVDs and ageing of population, may enhance the burden of HF greater than that in the Western populations [3-5]. There is an urgent need to have HF registries in the secondary, tertiary care centers and at the national level. The heart failure registry may help and provide us the detailed information related to incidence, prevalence, and aetiology of HF in India. In earlier studies from India, rheumatic heart disease has been the major cause of HF [3,4]. In later studies, CAD and cardiomyopathy have become major causes of HF in India [5,6]. The mechanisms of HF may be related to oxidative stress, mitochondrial and microRNA dysfunction, inflammation and disturbed myocardial substrate metabolism as observed in various studies [6,7]

In United States, HF is the third most common cardiovascular disease (CVD) affecting about 2 per cent of the population, or almost 5 million people [1]. The prevalence of HF increases with the age from less than 1 per cent in the 20-39 yr old age group to over 20 per cent in the people age 80 yr or older [1,2]. The lifetime risk of developing HF at the age of 40 yr is 11.4 per cent for men and 15.4 per cent for women. The hospital discharges for HF remained essentially unchanged from 2000 to 2010 in United States, with first-listed discharges of 1,008 000 and 1,023 000, respectively [1]. The deaths and disability due to HF

[*] Correspondence: Assist. Prof. Teodora Handjieva-Darlenska, MD, PhD. Department of pharmacology and toxicology. Faculty of Medicine, Medical University. 2 Zdrave Str.1431 Sofia, Bulgaria, *teodorah@abv.bg*

are also quite high in the USA [1]. In 2009, 1 in 9 death certificates (274 601 deaths) in the USA, mentioned HF and it was the underlying cause in 56410 of those deaths in 2009. The number of any-mentioned deaths attributable to HF was approximately as high in 1995 (287 000) as it was in 2009 (275 000). More than 7 million Europeans have congestive HF and it is a leading cause of hospitalization [1,2]. The major risk factors for HF are hypertension, coronary artery disease (CAD), dyslipidaemia, diabetes, obesity, genetic predisposition, viruses, and toxins [1-4]. The distinction of heart failure with reduced ejection fraction and with preserved ejection fraction has been clarified. In both situation s, clinical manifestations are important for the diagnosis of heart failure. The diagnosis of heart failure with preserved ejection fraction requires left ventricular enlargement and or diastolic dysfunction. However, in suspected heart failure, natriuretic peptide level in conjunction with echocardiography may be considered as important diagnostic methods. In patients with non-acute onset of heart failure, values less than 35 and less than 125 pg/ml for BNP and NT pro BNP make the diagnosis of heart failure unlikely [2].

A recent study was conducted to determine whether warfarin (with a target international normalized ratio of 2.0 to 3.5) or aspirin (at a dose of 325 mg per day) is a better treatment for patients in sinus rhythm who have a reduced left ventricular ejection fraction (LVEF) [8]. This study followed 2305 patients for up to 6 years (mean [±SD], 3.5±1.8).

The rates of the primary outcome were 7.47 events per 100 patient-years in the warfarin group and 7.93 in the aspirin group (hazard ratio with warfarin, 0.93; 95% confidence interval [CI], 0.79 to 1.10; P=0.40) showing no significant overall difference between the two treatments. However, in a time-varying analysis, the hazard ratio changed over time, slightly favoring warfarin over aspirin by the fourth year of follow-up (P=0.046).

Warfarin, as compared with aspirin, was associated with a significant reduction in the rate of ischemic stroke throughout the follow-up period (0.72 events per 100 patient-years vs. 1.36 per 100 patient-years; hazard ratio, 0.52; 95% CI, 0.33 to 0.82; P=0.005). The rate of major hemorrhage was 1.78 events per 100 patient-years in the warfarin group as compared with 0.87 in the aspirin group (P<0.001). The rates of intracerebral and intracranial hemorrhage did not differ significantly between the two treatment groups (0.27 events per 100 patient-years with warfarin and 0.22 with aspirin, P=0.82). A reduced risk of ischemic stroke with warfarin was offset by an increased risk of major hemorrhage. In conclusion, the choice between warfarin and aspirin should be individualized, however, among patients with reduced LVEF who were in sinus rhythm, there was no significant overall difference in the primary outcome between treatment with warfarin and treatment with aspirin.

In the recent trial, of the 1476 patients in the retrospective analysis, who were followed for a median of 2.15 years, 892 (about 60%) were prescribed aspirin at baseline. Of those, 91% received aspirin at 75 mg/day and the remainder received aspirin at higher dosages [9].

Apart from aspirin clopidogrel, was also given to 18.4% of the aspirin group, while 27.8% of them took added warfarin, and 2.1% received dual antiplatelets and warfarin. In this study, daily low-dose aspirin, defined as 75 mg/day, was followed by a 42% mortality reduction over several years in a cohort of patients participating in a heart-failure disease-management program [9].

Aspirin prolonged survival regardless of whether patients had a standard aspirin indication, such as ischemic heart disease, peripheral vascular disease, or stroke [9]. Both low- and higher-dose aspirin improved heart-failure hospitalizations, but there was no such survival benefit at dosages higher than 75 mg/day. These findings contrast with and go beyond prior studies of narrower heart-failure populations treated with aspirin.

Adjustment was made for age, sex, natriuretic-peptide levels, creatinine levels, heart-failure etiology, ischemic heart disease, atrial fibrillation, hypertension, diabetes, dyslipidemia, chronic obstructive pulmonary disease, peripheral vascular disease, and stroke. It seems that aspirin in above trials was given at two to four times the low dose of the current analysis, which indicates that treatment with low-dose aspirin may have a continuing role in secondary prevention of HF.

Table 1. Effects of antiplatelet agents on End-Point Hazard Ratios (95% CI) for low-dose vs high-dose aspirin vs none

End points	Low dose aspirin vs no aspirin	Low dose aspirin vs high dose aspirin	High dose aspirin vs no aspirin
Heart failure hospitalization	0.70 (0.54–0.90)	1.27 (0.70–2.30)	0.50 (0.27–0.92)
Mortality	0.58 (0.46–0.74)	0.57 (0.35–0.92)	0.98 (0.59–1.63)

In brief, it also suggests to a need for further prospective, trials of low-dose aspirin therapy in patients with HF. The findings in part solve the controversy on aspirin use in HF by presenting reassuring results on low-dose aspirin therapy in a clinical-practice.

Conflict of interest has not been declared by the authors.

Acknowledgements are due to International College of Cardiology and International College of Nutrition for logistic support to write this article.

References

[1] Go AS, Mozaffarian D, Roger VL et al. Heart Disease and Stroke Statistics 2013 Update. A report from the American Heart Association. *Circulation 2014; 129: e28-e292* Published online before print December 18, 2013, doi: 10.1161/01.cir.0000441139.02102.80.

[2] ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2012; 14: 803-869. doi: 10.1093/eurjhf/hfs105.

[3] Vakil RJ.A statistical study of 1281 cases of congestive cardiac failure or myocardial insufficiency in India. *Indian Physician*. 1949 Oct;8(10):281-9.

[4] Singh RB, Rai AN, Dube KP, Srivastav DK, Somani PN, Katiyar BC. Radioimmunoassay of serum digoxin in relation to digoxin intoxication . *Brit Heart J* 1975; 37:619-623.

[5] Huffman MD, Prabhakaran D. Heart failure: epidemiology and prevention in India. *Natl Med J India*. 2010;23:283-8.

[6] Kumar A, Singh RB, Saxena M, Niaz MA, Josh SR, Chattopadhyay P, Mechirova V, Pella D, Chopra R. Effect of carni Q-gel (ubiquinol and carnitine) on cytokines in patients with heart failure in the Tishcon study. *ActaCardiol* 2007;62:349-354.

[7] Hristova K, Singh RB, Fedacko J, Toda E, Kumar A, Saxena M, Baby A, Toru T , De Meester F, Wilson DW. Causes and risk factors of chronic heart failure in India. *World Heart J* 2013;5:13-20.

[8] WARCEF Investigators. Warfarin and Aspirin in Patients with Heart Failure and Sinus Rhythm. *N Engl J Med* 2012;366:1859-69.

[9] Bermingham M, Shanahan MK, O'Connell E, et al. Aspirin use in heart failure: Is low-dose therapy associated with mortality and morbidity benefits in a large community population? *Circ Heart Fail* 2014; DOI:10.1161/CIRCHEARTFAILURE.113.000132.

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Sofia Declaration for Prevention of Cardiovascular Diseases and Type 2 Diabetes Mellitus: A Scientific Statement of the International College of Cardiology and International College of Nutrition*

Krasimira Hristova[†*1], Daniel Pella[2], Ram B. Singh[3], Borislav D. Dimitrov[4], Hilton Chaves[5], Lekh Juneja[6], Tapan K. Basu[7], Lech Ozimek[8], Amrat K. Singh[9], Shanti S. Rastogi[10], Toru Takahashi[11], Douglas W. Wilson[12], Fabien De Meester[13], Sukhinder Cheema[14], Branislav Milovanovic[15], Harpal S. Buttar[16], Jan Fedacko[17], Ivo Petrov[18], Svetoslav Handjiev[19], Agnieszka Wilczynska[20], Manohar L. Garg[21], Ivy Shiue[22] and R. K. Singh[23]

[1]President of ICC and President of 7th ICCD and 17th WCCN, Sofia, Bulgaria & International College of Cardiology, Slovakia
[2]Executive Director, International College of Cardiology, Department of Sports Medicine, PJ Safaric University, Kosice, Slovakia
[3]Halberg Hospital and Research Institute, Moradabad, India
[4]Primary Care & Population Sciences, University of Southampton, Southampton, UK
[5]Federal University of Pernambuco, Recefe, Brazil
[6]Taiyo Kagaku Co. Ltd., Yokkaichi, Mie, 510 0844, Japan
[7]International College of Nutrition, University of Alberta, Edmonton, Canada
[8]Executive Director, International College of Nutrition, Edmonton, Canada
[9]Secretary General, International College of Nutrition, Neuron Hospital, Moradabad, India
[10]Diabetes and Endocrinology Center, Delhi, India
[11]Department of Nutrition, Graduate School of Human Environment Science, Fukuoka Women's University, Japan
[12]School of Medicine, Pharmacy and Health, Durham University, UK
[13]The Tsim Tsoum Institute, Krakow, Poland
[14]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada
[15]President, Neurocardiological Society, Neurocardiological Laboratory, Department of Cardiology, University Clinical Center Bezanijska Kosa, University of Belgrade, Serbia
[16]Department of Pathology & Laboratory Medicine, University of Ottawa, Faculty of Medicine, Ottawa, Canada
[17]Department of Internal Medicine, PJ Safaric University, Kosice, Slovakia
[18]President of Bulgarian Advisory Board, Bulgarian Society of Cardiology
[19]President, Bulgarian Society of Health, Nutrition and Foods, Sofia, Bulgaria
[20]The Tsim Tsoum Institute, Krakow, Poland
[21]School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, Australia
[22]School of the Built Environment, Heriot-Watt University and Department of Psychology, University of Edinburgh, Edinburgh, UK
[23]King George Medical University, Lucknow, UP, India

*To appear in small part in the journal *Nutrition* in 2014.

† Correspondence to: Dr. Krassimira Hristova, MD, PhD, FESC, FICC. University National Heart Hospital. Department of Noninvasive Functional Diagnostic and Imaging. 65, Konuivitsa str. Sofia, 1000, Bulgaria. tel +359 2 9211384. mobile: +359 8888 71353. e-mail: khristovabg@yahoo.com

Abstract

Background: There has been persistent emphasis from various health agencies including United Nations on the prevention of cardiovascular diseases (CVDs) and other chronic diseases. This review focusses on the emergence of CVDs and other chronic diseases as well as on modern strategies for their prevention.

Methods: A systematic and narrative review was conducted using such reference databases as MEDLINE (PubMed), Web of Science and EBSCO, with additional secondary sources and grey literature searching. Opinions of experts were also sought and discussions followed.

Results: The prevalence of primary risk factors for most chronic diseases is rapidly increasing in low and middle income populations due to the on-going economic development and progress. There is a decrease in such risk factors in the developed countries as due to education and adoption of preventive strategies result in a reduction in CVD mortality. Hypertension (5-10%), type 2 diabetes (3-5%) and CAD (3-4%) are very low in the adult rural populations of India, China, and in the African sub-continent which has less economic development. It seems that it is not poverty, but the lack of health education, possibly due to ineffective policies of national and local governments. In urban and immigrant populations of India and China, which are economically better off, NCDs are significantly higher than they are in some of the high-income populations. Health education and promotion of healthier lifestyle and behaviour appear to be important for prevention in such countries.

Conclusion: These findings may require modification of the existing American and European guidelines, proposed for the prevention of CVDs and other chronic diseases, in high-income populations.

Keywords: Diet, nutrition, hypertension, heart disease, type 2 diabetes, stroke

Introduction

The International College of Cardiology and the International College of Nutrition in their recent meetings in Sofia, 7th International Congress on Cardiovascular Diseases and 17th World Congress on Clinical Nutrition (Oct 24-26,2013, Sofia, Bulgaria, www.iccsk.bizpa.in), reiterated that treatment decisions for patients with obesity should be dictated by their overall level of risk due to primary risk factors rather than biological risk factors alone [1-6]. Obesity and poverty are the gateways for development of CVDs and other chronic diseases which have been recently highlighted by the World Health Organization and United Nations of Organization [7,8].

The International College of Nutrition and International College of Cardiology have been emphasizing that health education about the role of nutrition in the emergence of CVDs and other chronic diseases, is vital as evidence has accrued in the last three decades [1-6, 9,10]. Recently Clark has re-emphasized that NCDs area challenge to sustainable human development and no amount of money can control this epidemic [11]. Despite billions of dollars and a very high health budget, the epidemic of poor cardiovascular health behaviour continue to be common in USA because Americans may have wealth but there is no health advantage compared to 15 other western countries [12]. In a recent report of the American Heart Association [13], after adjustment, population attributable fractions for CVD mortality were as follows; for high blood pressure, 1: 40.6% (95% confidence interval [CI], 24.5–54.6); for smoking 13.7% (95% CI, 4.8–22.3); for unhealthy diet 13.2% (95% CI, 3.5–29.2); for sedentary behaviour 11.9% (95% CI, 1.3–22.3) for high glucose; 8.8% (95% CI, 2.1–15.4). These findings further confirm the old hypothesis that emergence of NCDs may have a sequence during transition from under-nutrition and poverty to affluence; Overweight and central obesity come first in conjunction with deficiency of certain lifestyle related biomarkers having adverse effects on emergence of NCDs. This sequence of development of NCDs may be altered by learning and regular practice of methods of prevention by alteration in public health policies for achieving total health including social, mental and spiritual health, apart from the physical health and environmental health [14].

How This Document Differs from Others

This document is slightly different from other documents proposed by other agencies because we emphasize more on knowledge and practice of health education and the alteration in government policies to provide physical activity and health foods at affordable costs to citizens in the developing and newly industrialized countries. If, and only if, we do

not modify the UNO and WHO advice and follow North America and Western Europe, we would make the same mistakes which the western world has done which is to buy health education and health behaviour from health insurance companies [11-13].

There is a need for the governments of all countries to collaborate between their Ministry of Health, Ministry of Education, Ministry of Sports, Ministry of Food and Nutrition and Ministry of Agriculture in order to produce policies that change the health behaviour of the populations rather than targeting secondary risk factors of NCDs [14]. It should be re-emphasized that poverty is not the cause of emergence of NCDs but poor health behaviours are actual causes of deaths due to NCDs [15].

In this connection, industry and institutions need to provide information on physical activity, health foods; stress relief by yoga and meditation, tobacco and alcoholism cessation, moderate wine drinking, decreasing pollution should be encouraged by giving tax relief by the governments. These measures may cause an apparent decrease in the income to governments but would be cost effective in the long run by decreased health care budgets and by increasing work efficiency of every country. There is a need to re-emphasize that risky health behaviours continue to be common in most high income countries during nutritional transition and economic development from Homo sapiens to *Homo economicus* [15-17].

A recent survey to assist in targeting the adults who undertake risky behaviours, showed that they know about their health behaviours which are not optimal but are worried about their health [18].

This situation can arise when health foods and exercise are expensive and people are not motivated to change their health behaviour due to poor social, mental and spiritual health.

United Nations Organizations and World Health Organization

In September 2000, the United Nations made a Millennium declaration and set 8 Millennium development goals to focus international development efforts on decreasing global poverty by 2015 [19]. Of the 8 Millennium Development Goals, 3 goals directly address health of the people and populations, without mention of NCDs, health and general education but for human development. This epidemic of NCDs offered us an opportunity to initiate action to counter growing epidemics of cardiovascular diseases (CVD) on both sides of the Atlantic, although it has been associated with great economic burden [10-13]. There was a declining trend in CVD in the Western world after 1968, but obesity continued to increase, resulting in an increase in the metabolic syndrome in both developed and developing economies leading to increased burden and mortality due to NCDs [14-21]. Rosenbaum and Lamas considered it a "Slow-Motion Disaster" in the New England Journal of Medicine 2011 [21]. However, a scientific statement from the International College of Cardiology, Columbus Paradigm Institute and the International College of Nutrition proposed that globalization of dietary wild foods can protect against cardiovascular disease and all-cause mortalities [22].

Such statements have to be made again and again to educate the food industry and governments to increase the availability of health foods at affordable cost.

The authors of *The Sofia Declaration* are delighted to congratulate the planners of The United Nation High-Level Meeting (UN HLM) on deaths and disability due to NCDs held in September, 2011 which has been very exciting [20].The world's Heads of States and Health Ministers attended the meeting, creating a unique opportunity to advance globally the prevention and treatment of NCDs. The burden of death and disability attributable to CVDs and other chronic diseases is rising in all middle- and high-income countries, because of the rapid changes in the lifestyle patterns [23-25]. Millions of deaths occur every year due to lack of health education and poor health policies [1-8, 23-25]. NCDs are a great challenge to health care experts and governments and appear to be an underlying cause for poverty as well as threat to human, social, and economic development [23-25].

The risk of cardiovascular disease (CVD); hypertension, stroke CAD and other chronic diseases, as well as risk of risk factors, is changing dynamically around the world [21-25].The 36·1 million deaths per year as a result of NCDs represent almost two out of three deaths per year worldwide. 22·4 million of these

deaths arise in the poorest countries, and 13·7 million in high-income and upper-middle-income countries [23]. Approximately, two-thirds (63%) of premature deaths in adults (aged 15–69 years), and three of four of all adult deaths are attributable to NCDs. In the South Asian sub-continent, where type 2 diabetes and CAD are highly prevalent, it is clearly apparent that people of South Asian descent, around the globe have the highest rates of premature CAD, with clinical manifestations occurring about 10 years earlier than in other populations [24, 25]. In India, majority of the deaths occur due to infectious diseases (41.1%, n=915) in the urban population [24, 25]. NCDs as the cause of deaths(60.2%) were; circulatory diseases (29.1%, n=646) (heart attacks (10.0%), stroke (7.8%), valvular heart disease (7.2%,n=160), sudden cardiac death and inflammatory cardiac disease, each, 2.0%,n=44), type 2 diabetes (2.2% 49),malignant neoplasm (5.8%, n=131), injury (14.0%,n=313) including accidents; fire and falls and poisonings, were also quite common causes of death. Miscellaneous causes of deaths were noted in 9.1%, (n=202).

Circulatory diseases as the cause of mortality were significantly more common among higher social classes 1-3 than in lower social classes 4 and 5 who died more often, due to infections. Heart attacks, stroke, hypertension, diabetes and obesity were significantly more common among higher social classes 1-3 compared to class 3 and 4 but tobacco intake showed only minor differences in various classes. In the study, reported that apoA1, the primary protein that makes up approximately 75% of HDL particles, is oxidized by myeloperoxidase (MPO) at Trp72, and such oxidation impairs the cardio-protective functions of HDL making it dysfunctional and proinflammatory [33]. It is possible that it may be an unknown target of behavioural risk factors tobacco, sedentary behavior, transfat and stress.

Nutrition in Transition and Development of NCDs

The world population has been under transition from Homo sapiens to homo economicus communities after the ancient agricultural revolution, some 10,000 years ago, when humans started farming and storing of foods [17]. They continued to eat Palaeolithic foods along with whole grains obtained from their farming and animal foods by hunting associated with enormous physical activity [17]. The major changes in the risk factors for CVDs have mainly occurred in the last 100 years after 1910 due to industrialization and urbanization resulting into rapid changes in the diet and lifestyle with emergence of risk factors of CVDs first in the developed countries and then in all other countries [1-9] (Table 1). The major primary risk factors for CVDs including hypertension and type 2 diabetes mellitus are identical to those of other chronic diseases, including cancer and chronic respiratory diseases where pollutants, tobacco, allergens and carcinogens are more important apart from other lifestyle factors [7, 8,16, 27-47].

There is some emphasis on global mental health and associated diseases such as depression [39, 40]. In most developed countries, the emphasis is on primary risk factors such as nutrition, tobacco, alcoholism and physical activity is minimal and the role of diet and nutrients are not given due consideration due to growing interest of the *Homo Economicus* community in the capitalist culture, where the sole aim is to enhance profit by increased sale of drugs and stents [42-44]. Implications of new insurance coverage for access to care, cost-sharing, and reimbursement in the United States, indicate some fractional encouragement [43].

Unfortunately the whole world is following the culture and systems prevalent in these countries, whether it is housing, consumer durables, automobiles, indoor recreations, fashion, late night awakening, eating and drinking and empowering with weapons with little consideration for no tobacco, moderate alcohol, regular physical activity, meditation which are rapidly adopted in high-income countries [44-46].

The majority of the risk factors and health problems benefit from continuity of care; strengthening primary health-care services to deliver long-term care for all disabling illnesses will benefit patients with mental health issues and people with other NCDs, e.g., kidney disease and musculoskeletal disorders [10-12, 24-30].

Table 1. Nutrition in transition and emergence of non-communicable diseases in developing countries

Homo sapiens diet patterns	Pattern 1: Hunter-gatherers	Pattern 2: Food Scarcity-Poverty	Pattern 3: Receding Food Scarcity & Poverty	Pattern 4: More food, less exercise- *Homo economicus*	Pattern 5: Healthy Behavior- *Homo modestis*
Nutrition profile / diet	Plants, low-fat wild animals, diet diversity by collecting foods	Cereals predominant, diet less varied	Fewer starchy staples; more fruit, vegetables, animal protein; low variety continues	More fat (animal products, trans fat, w-6 fat), sugar, processed foods; less fibre, less w-3 fat and flavonoids	Higher-quality fats, reduced refined carbohydrates, more whole grains, fruit, vegetables rich in w-3 and flavonoids
Nutritional status	Robust, lean population; few nutritional deficiencies	Children and women suffer most from low fat intake, nutritional- deficiency disease emerge, stature declines	Continued MCH[1] nutrition problems, many deficiencies disappear, weaning diseases emerge, stature grows	Obesity, problems for elderly (osteoporosis, fractures etc.), type 2 diabetes, hypertension, stroke, heart attack, brain degeneration, psychological disorders, cancer	Reduction in body fat and obesity, and NCDs, improvement in bone health; epigenetic modulation and transgenerational epigenetic inheritance -natural selection.
Economy	Hunter-gatherers	Agriculture, animal husbandry, homemaking begin; shift to monocumono cultures	Second agricultural revolution (crop rotation, fertilizer), Industrial Revolution, women join labour force	Fewer jobs with heavy physical activity, service sector and mechanization, household technology revolution	Service sector mechanization and industrial robotisation dominate, increase in leisure exercise offsets sedentary jobs
Household	Primitive, onset of fire	Labour-intensive, primitive technology begins (clay cooking vessels)	Primitive water systems, clay stoves, cooking technology advances	Household technology mechanizes and proliferates	Significant reduction in food preparation costs as a result of technologic change
Income and assets	Subsistence, primitive stone tools	Subsistence, few tools	Increases in income disparity and agricultural tools industrialization	Rapid growth in income and income disparities, technology proliferation	Decrease in income growth, increase in home and leisure technologies
Professional skill/Education	Hunting	Stock breeding , cultivation	Industry, intensive agriculture	Processed unhealthy foods increased	Functional foods availability increases
Demographic profile / Mortality	Low fertility, high mortality, low life expectancy	Age of Malthus; high natural fertility, short life expectancy, high infant and maternal mortality	Mortality declines slowly, then rapidly; fertility static, then declines; small, cumulative population growth, which later explodes	Life expectancy hits unique levels (ages 60–70), huge decline and fluctuations in fertility (e.g., post-war baby boom)	Life expectancy extends to ages of 70 and 90 years, disability-free period increases
Age structure	Young population	Young, very few elderly	Chiefly young, shift to older population begins	Rapid decline in fertility, rapid increase in proportion of elderly person	Increases in the proportion of elderly >75 years of age
Housing	Rural, low density	Rural, a few small, crowded cities	Chiefly rural, move to cities increases, international migration begins, megacities develop	Dispersal of urban population decrease in rural green space	Lower-density cities rejuvenate, increase in urbanization of rural areas encircling cities
Food processing	Non-existent	Food storage begins	Storage processes (drying, salting) begin, canning and processing technologies emerge, food refining and milling	Numerous food- transforming technologies	Technologies create functional foods and food constituent substitutes (e.g., macronutrient substitutes)

[1] MCH denoted maternal and child health. As modified from Popkin et al (2006), References 20,38,42.

Health education about pathogenesis of various risk factors early in life emphasizes the importance of prevention of NCDs during the lifetime of an individual [41-45]. It is important to educate girls and women before conception about the health and nutrition to achieve better health during pregnancy which may lead to prevention of NCDs in offspring [45-48]. In developing countries, people and populations having chronic respiratory diseases are highly predisposed to deaths due to tuberculosis and pneumonia [49]. Presence of HIV increased the risk of CVDs and tuberculosis [50]. Public health benefits of strategies to reduce green-house gas emissions, indoor pollutions, and urban land transport have to be emphasized for prevention of NCDs [51-53]. Health effects and financial costs of such strategies to reduce salt intake and tobacco are known in both middle and high income countries [53-55]. Dietary changes and physic al activity have been found to tackle obesity in all the countries of the world to prevent all the NCDs [56-62].

In brief, in respect of such healthcare services, Figures 1 and 2, demonstrate the potential for the 'rise and fall' of Homo economicus' with increasing capital generation and man's changing behavioural patterns in a growing urban civilization and environment leading to the increasing development and expression of risk factors and ensuing global consequences of 'epidemic' proportions of morbidity and mortality of NCDs: though communicable diseases are also pressing problems, globally. Tobacco use, including exposure to second-hand smoke, diets high in refined carbohydrates, saturated fat, w-6 fat and trans fat, salt, and sugar, low in w-3 and monounsaturated fatty acids (MUFA) and antioxidants and environments that prevent physical activity, and increase alcoholism and mental stress are important behavioural risk factors [1-8,23-26,53-55].

These risk factors are the results of industrialization, urbanization and globalization which increase the risk of biological risk factors; obesity, increased blood pressure and concentrations of glucose and lipids and proinflammatory cytokines are now common in the poorest countries, and are rising rapidly [32-39]. Underlying these biological risk factors are socioeconomic determinants such as poverty, inequality, unemployment, social instability, unfair trade, and global imbalances, which appear to be the root causes of the pandemic of NCDs [21-23] (Figure 2).

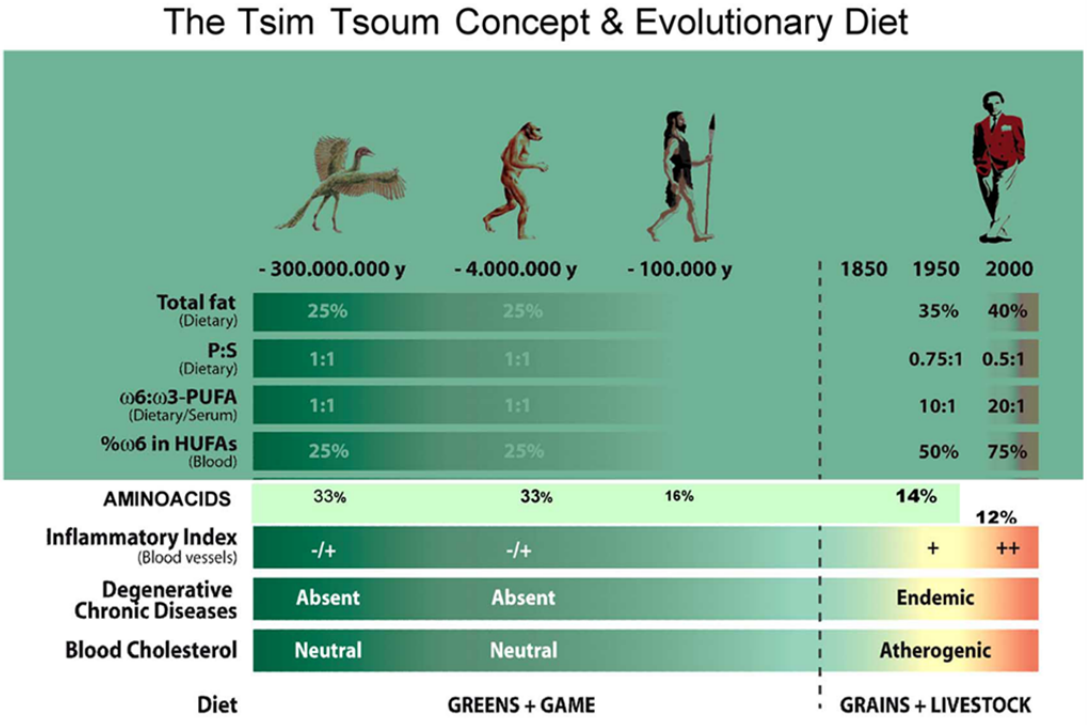


Figure 1. Nutrient intake during Palaeolithic period and in modern men.

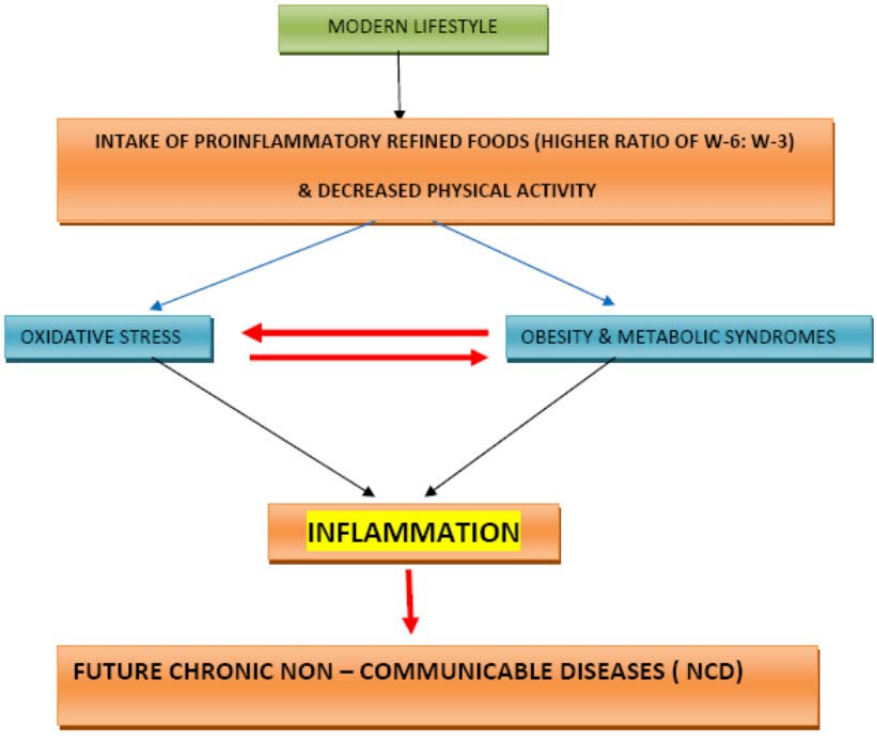


Figure 2. Lifestyle and development of diseases.

It is important to emphasize the vascular variability disorders, night shift work, disruption in sleep and chrononutrition leading to chronic gastrointestinal diseases that are more recent problems which need emphasis in the prevention of NCDs [58-62]. For instance, the physiologic monitoring facilitated by recent technological advances can serve for self-surveillance, so that intervention can be promptly instituted when needed. A chronobiologic analysis of data collected around the clock interpreted in the light of time-specified reference values specified by gender and age finds abnormalities in the variability of blood pressure and heart rate, known as vascular variability disorders (VVDs) [61].

As these may occur within the physiologic range, they serve for true primary prevention, before there is target organ damage. VVDs have been associated with an increase in cardiovascular disease risk in several outcome studies [87]. They raise the risk from about 8% in patients with uncomplicated MESOR-hypertension to over 35% and 50% when MESOR-hypertension is accompanied by one or two additional VVDs, respectively. Some VVDs, such as CHAT (Circadian Hyper-Amplitude-Tension, a condition characterized by too large a circadian variation in blood pressure) can be treated, sometimes by only changing the scheduling of treatment administration, a practice that can cut to less than half the number of strokes and other all cardiovascular events [88].

Treatment timing should be optimized for the individual patient so as to avoid trading one VVD (MESOR-Hypertension) for another (CHAT) associated with an even larger risk [89]. Much attention has recently been also paid to risks associated with shift-work, notably in relation to cancer. Whereas irregular rest-activity schedules may raise some issues in their own right, the possibility of optimizing schedule shifts should be revisited, as in several studies in the experimental laboratory, some schedule shifts were shown to be beneficial rather than harmful (for review, see [90]).

Burden of Noncommunicable Diseases

The total world population in 2012 was above 7.0 billion which would increase to 7.9 billion by the year 2025. Adult population aged 20-79 years, in the world

was 4.1 billion, in the year 2007, which would increase to 5.2 billion by the year 2025. Of the total world's population, 80% of them live in developing countries and 80% of all CVD deaths occur in lower- and upper-middle income countries [18,42,43].

As mentioned earlier, 36·1 million deaths per year as a result of NCDs represent almost two of three deaths per year worldwide. Of these deaths, 22·4 million arise in the poorest countries, and 13·7 million in high-income and upper-middle-income countries [18]. Figures show mortality data (millions) from the World Bank for income groups illustrating three main points: a) that mortality from NCDs relative to injury and communicable diseases increases from poverty to affluence; b) by far the largest group for NCD mortality is in the lower-middle income group; and c) it is 'b' who experience a higher mortality due to injury and communicable disease which are very important but outside the scope of this report. However, when age-standardized mortality data is expressed for the same income groups by selected countries, it is clear that a similar order from poverty to affluence prevails though other factors are important such as health care services provision, health education, quality of housing, urbanization, crops and animal husbandry, education, etc., will all have an impact on mortality quite apart from communicable diseases.

NCDs, principally CVD, cancer, chronic respiratory disease and diabetes mellitus caused 35 million deaths, which were 60% of total deaths in the year 2005. CVDs are the leading chronic diseases, with 17 million deaths. Deaths due to diabetes are usually recorded as being deaths due to heart disease, stroke and renal failure [17,18]. Majority of these deaths (80%) occurred in low- and middle-income countries. In one recent study [22, 23], all-cause mortality were; infectious diseases (41.1%) and NCDs as the cause of death were common among 60.00% of the victims dying due to various causes.

According to the Registrar General of India, in the year 1994 to 1998, trends indicate that there has been a significant decline of proportionate deaths from infectious diseases from 22% to 16%. However, mortality from cardiovascular disease (CVD) increased from 21% to 25% which is lower than the death rate of 29.1 reported by Singh et al in 2005 [13, 22, 23].

Major NCDs are responsible for 85% of deaths and 70% of the disease burden in Europe and North America [13,18,33-35]. The costs for the care of the consequences of NCD present an enormous burden for the economy of the country in addition to the human suffering: even in those more affluent Member States where one sees downward trends in NCD mortality (primarily CVD, cancer, chronic obstructive pulmonary disease (COPD) and diabetes) [33-39]. Chronic disease places an enormous health and economic burden on the population of all Member States of the WHO European Region (41). Multiple risk factors management is a critical area in the prevention and therapy of NCD and it has been shown to be effective in reducing mortality and disability mainly in CVD. It is well documented that NCDs have a significant impact on national economies by disabling and killing the working-age population [12,13,42].

The health systems of most developed countries are not adequately structured to respond to these emerging needs. The dissemination of information on the early diagnosis, treatment, and prevention of multiple risk factors in disease in many countries, has multiple barriers. These barriers are linked to a lack of integrated country-specific policies. For instance, in many countries, 24-hour ambulatory blood pressure (ABPM) monitoring may help the earliest possible and most precise diagnosis of hypertension and vascular variability syndromes (VVSs) as risk factors for cerebro- and cardio-vascular diseases. As an example of such a good practice, ABPM has been recently recommended for the confirmation of the diagnosis of hypertension in the UK and can be feasibly integrated into such clinical prediction rules as ABCD2 or CHADS2 scores to predict the risk of stroke after transitory ischaemic attack (TIA) or atrial fibrillation, respectively [82-86]. Majority of the NCDs (as CVD, obesity, diabetes, COPD and some tumours) burden on lives and economy can be decreased by appropriate integrated approaches to health policies applied to: individual risk reduction (aimed at high-risk individuals), population risk reduction (aimed at social determinants), rational use of health services (by empowering primary health care), and referral system support and help from non-government organizations.

Diet and Risk of Noncommunicable Diseases

Our genes appear to be similar to the genes of our ancestors during the Palaeolithic period 40,000 years ago, the time when our genetic profile was established [59-62]. The affluent man appears to live in an environment that completely differs from that which his genetic constitution was originally selected. Unfortunately, during the last 100 years, our dietary intakes and lifestyles have changed significantly, causing increased intakes of refined carbohydrates, saturated fatty acids (SFA), transfat, and w-6 fat and decreased intakes of ω-3 fatty acids, from grain-fed cattle tamed at farm houses rather than meat from hunted free-roaming animals [61]. The food and nutrient intakes among hunter-gatherers during the Palaeolithic period comprised mainly fruits, vegetables, seeds, whole grains, egg, fish and wild animal meat which were low in w-6 and high in w-3 fatty acids along with antioxidants, vitamins and minerals and amino acids. In comparison to modern diets, there is a marked reduction in consumption of ω-3 fatty acids, vitamins, minerals and proteins, and a significant increase in intakes of carbohydrates (mostly refined), fats (saturated and unsaturated trans fats, linoleic acid) and salt in the modern diets [2-4,6-10, 59-62]. The high cardiovascular mortality in Eastern Europe and all other middle income countries has often been attributed to poor diet, but individual-level data on nutrition in all of the countries are generally not available. In a review on food intakes [59], a few subjects met the WHO recommended intakes for complex carbohydrates, pulses or nuts; intakes of saturated fatty acids, transfat and w-6 fat, sugar and salt were too high. Only 16% of Polish subjects met the WHO recommendation for polyunsaturated fat intake. Consumption of fruits and vegetables was lower than recommended by WHO (>400g/day), especially among those Russian subjects who were assessed during the low intake season.

Various health agencies such as WHO, International College of Cardiology and International College of Nutrition have been emphasizing for the last two decades about the role of diet and physical inactivity in the development of CVDs and other chronic diseases [2-9]. Recent studies indicate that a prudent dietary pattern, similar to a Mediterranean-style diet may be protective against CVDs and other chronic diseases [59,60]. Cohort studies and meta-analysis of studies showed that a fruit and vegetable enriched diet can protect against myocardial infarction and modulate microvascular function [60]. In a meta-analysis on the role of diet in metabolic syndrome [63], the authors conducted a systematic review and random effects of epidemiological studies and randomized controlled trials, including 50 original research studies (35 clinical trials, 2 prospective and 13 cross-sectional), with 534,906 participants, were included in the analysis. The combined effect of prospective studies and clinical trials showed that adherence to the Mediterranean diet was associated with reduced risk of metabolic syndrome (log hazard ratio: -0.69, 95% confidence interval [CI]: -1.24 to -1.16). Additionally, results from clinical studies (mean difference, 95% CI) revealed the protective role of the Mediterranean diet on components of metabolic syndrome, like waist circumference (-0.42 cm, 95% CI: -0.82 to -0.02), high-density lipoprotein cholesterol (1.17 mg/dl, 95% CI: 0.38 to 1.96), triglycerides (-6.14 mg/dl, 95% CI: -10.35 to -1.93), systolic (-2.35 mm Hg, 95% CI: -3.51 to -1.18) and diastolic blood pressure (-1.58 mm Hg, 95% CI:-2.02 to -1.13), and glucose (-3.89 mg/dl, 95% CI:-5.84 to-1.95). The results from epidemiological studies also confirmed those of clinical trials. It is possible that this dietary pattern can be easily adopted by all population groups and various cultures and cost-effectively serves for primary and secondary prevention of CVDs and other chronic diseases.

Sofi et al. [64] demonstrates the lower risk associated prospectively with a Mediterranean diet which is well presented and heterogeneity amongst the selected studies is very low as seemingly is any publication bias: this study is well conducted. Sofi et al aimed to conduct meta-analysis of cohort prospective studies that investigated the effects of adherence to the Mediterranean diet on health status. The study showed that a 2-point increase in adherence to the Mediterranean diet was associated with a significant reduction of overall mortality [relative risk (RR) = 0.92; 95% CI: 0.90, 0.94], cardiovascular incidence or mortality (RR = 0.90; 95% CI: 0.87, 0.93), cancer incidence or mortality (RR = 0.94; 95% CI: 0.92, 0.96), and neurodegenerative diseases (RR =

0.87; 95% CI: 0.81, 0.94). The meta-regression analysis showed that sample size was the most significant contributor to the model because it significantly influenced the estimate of the association for overall mortality. In conclusion, this updated meta-analysis confirmed, in a larger number of subjects and studies, the significant and consistent protection provided by adherence to the Mediterranean diet in relation to the occurrence of major chronic degenerative diseases.

It seems that the majority of the epidemiological studies indicate that a prudent dietary pattern characterized with fruit, vegetable, legume and whole grain intake appears to be protective [59-66]. In one cross-sectional survey among 6940 subjects, above 25 years of age, fruit, vegetable and legume intake were inversely associated with risk of pre-hypertension and hypertension in five Indian cities [31]. This study was conducted during 1993 to 1996 in India when w-6/w-3 ratio of fatty acids of the diet was increasing due to increased consumption of sunflower oils, soya bean oil and corn oil. A meta-analysis of cohort studies quantitatively assessed the relation between fruit and vegetable intake and incidence of CAD which reported relative risks (RRs) and corresponding 95% confidence interval (CI) of CAD with respect to frequency of fruit and vegetable intake (66). A total of 278 459 subjects (9143 CAD events) were included, with a median follow-up of 11 years. The individuals who had less than 3 servings/day of fruit and vegetables, the pooled RR of CAD was 0.93 (95% CI: 0.86–1.00, P=0.06) while those with more than 5 servings/day, the RR was 0.83 (0.77–0.89, P=0.0001).This meta-analysis of prospective cohort studies demonstrated that increased consumption of fruit and vegetables, less than 3 to more than 5 servings/day is related to a 17% reduction in the risk of CAD. A lower increase in the intake to 3–5 servings/day was also associated with a smaller and borderline significant reduction in CAD risk. These results provide evidence supporting that 5 or more servings per day of fruit and vegetables, are needed to protect from CVD. The role of Mediterranean diet on blacks and Hispanics in the United States are lacking, particularly in relation to stroke [67]. The Northern Manhattan Study determined stroke incidence and risk factors among 2568 participants (mean and SD age of participants was 69 and10 y respectively; 64% were women; 55% Hispanic, 21% white, and 24% black). A higher score on a0–9 scale represented increased adherence to a Mediterranean diet rich in fruit, vegetables, whole grains, fish, and olive oil. The relation between the Mediterranean diet score and risk of ischemic stroke, myocardial infarction (MI), and vascular death was assessed with Cox models, with control for sociodemographic and vascular risk factors were as follows: 0–2 (14%), 3(17%), 4 (22%), 5 (22%), and 6–9 (25%). Over a mean follow-up of9 y, 518 vascular events accrued (171 ischemic strokes, 133 MIs, and 314 vascular deaths). The Mediterranean diet score was inversely associated with risk of the composite outcome of ischemic stroke, MI, or vascular death (P-trend = 0.04) and with vascular death specifically (P-trend = 0.02). Moderate and high Mediterranean diet scores were marginally associated with decreased risk of MI. There was no independent association with ischemic stroke but higher consumption of a Mediterranean diet was associated with decreased risk of vascular events.

In a large, prospective, observational study [68] involving 72,113 female nurses who were free of CAD, stroke, diabetes, and cancer, factor analysis identified 2 dietary patterns from data collected on serial food frequency questionnaires. One pattern, called prudent, was characterized by a high consumption of vegetables, fruit, legumes, fish, poultry, and whole grains. The other pattern, called Western, corresponded to a high consumption of red meat, processed meat, refined grains, French fries, sweets, and desserts. Individuals were classified by their level of adherence to both the prudent diet and the Western diet. After baseline data collection in 1984, follow-up lasted 18 years, during which time 6011 deaths occurred (3139 [52%] as a result of cancer; 1154 (19%) resulting from cardiovascular disease (CVD); and 1718 (29%) resulting from other causes).There was a 17% lower risk of total mortality among those who were most adherent to the prudent diet (highest versus lowest quintile of adherence), a 28% lower risk of CVD mortality, and 30% lower mortality from non-CVD, non-cancer causes. Cancer was not associated with the inverse prudent dietary pattern. A comparison of the highest and lowest quintiles of adherence showed that consumption of the Western diet was associated with increased total mortality (21%), CVD mortality (22%), cancer

mortality (16%), and mortality from non-CVD, non-cancer causes (31%). Hence, except for cancer, risk relationships for the prudent and Western dietary patterns appear to be the inverse of each other: Mortality thus was increased as adherence to the prudent diet decreased and adherence to the Western diet increased.

The Greek Epic Prospective cohort study [69] investigated the relative importance of the individual components of the Mediterranean diet in generating the inverse association of increased adherence to this diet and overall mortality. The Greek segment comprising of 23 349 men and women, not previously diagnosed with cancer, CAD, or diabetes, with documented survival status until June2008 and complete information on nutritional variables and important covariates at enrolment. After a mean follow-up of 8.5 years, 652 deaths from any cause had occurred among 12 694 participants with Mediterranean diet scores 0-4 and 423 among10 655 participants with scores of 5 or more. After potential confounders were controlled, higher adherence to a Mediterranean diet was associated with a statistically significant reduction in total mortality (adjusted mortality ratio per two unit increase in score 0.864, 95%confidence interval 0.802 to 0.932). Moderate ethanol consumption23.5%, low consumption of meat and meat products16.6%, high vegetable consumption 16.2%, high fruit and nut consumption 11.2%, high monounsaturated to saturated lipid ratio 10.6%, and high legume consumption 9.7% were individual components of the Mediterranean diet contributing to this association. The contributions of high cereal consumption and low dairy consumption were minimal, whereas high fish and seafood consumption was associated with a non-significant increase in mortality ratio. It is possible that moderate consumption of ethanol, low consumption of meat and meat products, and high consumption of vegetables, fruits and nuts, olive oil, and legumes were highly protective and minimal contributions were found for cereals and dairy products, possibly because they are heterogeneous categories of foods with differential health effects, and for fish and seafood which are consumed less often in Greece.

The INTERHEART study, involving participants from 52 countries [70] examined the relationship between dietary patterns and risk of acute coronary syndrome (ACS). Consistent with previous studies in single within-population cohort studies, the authors found an inverse association between the prudent pattern score and risk of ACS and a significant positive association between the Western pattern score and increased risk of ACS. No association of Oriental diet with risk of ACS was reported. A dietary risk score based on 7 food items on the food-frequency questionnaire (meat, salty snacks, fried foods, fruits, green leafy vegetables, cooked vegetables, and other raw vegetables) was constructed by the authors. The investigators found that a higher score, indicating a poor diet was strongly associated with ACS risk and the subjects in the highest quartile of the score had nearly 2-fold increased risk, even after adjustment for established coronary risk factors. On the basis of an arbitrary cut-point of the score (top 3 quartiles versus the bottom quartile), the investigators estimated that 30% of myocardial infarction (MI) could be explained by unhealthy diets worldwide.

The INTERHEART study is the first large study to quantify eating patterns in all geographic regions of the world. It provides evidence that despite different food habits in various populations, reproducible patterns can be found in diverse regions of the world. These findings are important because there has been a concern that dietary patterns derived through a data-driven approach such as principal components analysis (PCA) may be highly unstable and non-reproducible because of very different eating habits in different populations.

In the dietary-patterning analysis, habitual intake patterns are typically quantified by statistical methods such as factor or cluster Analysis or diet-quality indexes based on prevailing dietary recommendations or healthy traditional diets e.g., the Mediterranean diet, the Japanese diet and the Indo-Mediterranean diet [59-73]. The PCA is commonly used to define dietary patterns using food consumption information to identify common underlying dimensions (factors or patterns) of food intake. The method aggregates specific food items based on the degree to which these food items are correlated with each other. A summary score for each pattern is then derived and can be used to examine relationships between various eating patterns and outcomes of interest such as CAD, diabetes mellitus, stroke and other chronic diseases.

Earlier validation studies found that 2 major patterns (the prudent and Western patterns) identified through PCA of food consumption data assessed by food frequency questionnaires were reproducible over time and correlated reasonably well with the patterns identified from diet records.

The consistent association observed between the Western or unhealthy dietary pattern (high in animal products, salty snacks, refined starches and sugar and fried foods and low in fruits and vegetables) and ACS risk indifferent regions of the world from the INTERHEART study and other studies as well as in our study, provide consistent evidence of the adverse effects of globalization on human nutrition and NCDs risk. However, this evidence is indirect because these studies did not specifically assess the impact of global trade and marketing on food consumption patterns across different countries [63-70]. Despite this weakness, most recent studies suggest that the current trend of dietary convergence toward a typical Western diet characterized with high w-6/w-3 ratio of fatty acids, and low nutrient density, is likely to play a role in the globalization of obesity and NCDs [59-70].

The cohort studies provide an association of diet with risk of CVDs and deaths which do not provide a proof that is the cause of the problem. Therefore, randomized, controlled intervention trials are necessary to provide scientific rigour that diet has a role in the prevention of NCDs [71-78]. Intervention trials, using the whole diet approach so far conducted are also in line with this epidemiological evidence. The effect of Palaeolithic style diet was examined in patients (n=204 intervention group, n=202 control group) with acute coronary syndromes, which showed significant decline in total cardiac events as well as in total mortality after 6 weeks and the benefit continued after one year [71,72]. Further follow up for 2 years in this study [73] is different from the published work, because it places emphasis on the Palaeolithic dietary patterns and ALA content of the diet which is probably responsible for the significant greater survival in the intervention group compared to control group. Dietary patterns before entry to the study showed higher w-6/w-3 ratio of 32.5 in the diets of both the groups. Intervention group A was advised a Palaeolithic style diet with w-6/w-3 fatty acid ratio of 4.3 compared to standard diet group with ratio of 20. Dietary adherence was excellent. After a follow up of 2 years, total mortality was significantly declined in the Palaeolithic style diet group compared to control group. The mortality was lowest among subjects with w-6/w-3 ratio of less than 5 which showed graded increase with increase in the fatty acid ratio in both the groups. The diet and re-infarction trial [74] showed that modest intake of fish, 2 servings per week can cause 29% decrease in total mortality and cardiovascular mortality. Since no benefit was observed in nonfatal infarction, the authors concluded that w-3 fatty acids may have prevented ventricular fibrillation by altering cardiomyocyte cell membrane phospholipids.

In the Lyon Diet Heart Study [75], 605 patients who had a myocardial infarction were randomly assigned to a 'Mediterranean-style' diet or a control diet resembling the American Heart Association Step I diet. The Mediterranean diet model supplied 30% of energy from fats and < 10% of energy from saturated fatty acids, whereas the intake of 18:3 (n-3) (α-linolenic acid) provided >0.6% of energy. After a mean follow-up of 27 months, the risk of new acute myocardial infarction and episodes of unstable angina was reduced by ~ 70% by the Mediterranean diet. Moreover, total mortality was also reduced by 70%. Long-term follow-up for 4 years also showed that the beneficial effects of diet were continued. Singh et al [76] tested an 'Indo-Mediterranean diet' in 1000 patients in India, with existing coronary disease or at high risk for coronary disease. Half of the patients (n=499 vs. 501) were administered a diet rich in fruits, vegetables, whole grains, walnuts, mustard and soy bean oil as a source for w-3 fat and the rest i.e. 501 patients, were advised to take prudent diet advised by the National Cholesterol Education Program Step 1 diet in 1988. At the end of 2 year follow-up, the Palaeolithic style diet group consumed significantly more fruits, vegetables and legumes than did the control group (537± 127 vs. 231±19 g/day, $p<0.001$) as well as more mustard and soy bean oil (31±6.5 vs. 15.2±5.5g/day). The mean intake of ALA was over two fold greater in the Palaeolithic style diet group compared to control group (1.8±0.4 vs. 0.8±0.2 g/day, $p<0.001$). The w-6/w-3 ratio of fatty acids was slightly higher at baseline in the intervention group than in the control group (39±12 vs. 34±10) yet both these figures are extremely high, reflecting a diet with a very high w-6 content yet low w-3 (50). At the end

of two years follow up, this ratio showed a marked decline in the intervention group, which was greater than that observed in the control group consuming control diet (9.1 ± 12 vs. 21±10,p<0.001). The study end points were; significant decline in the total cardiac events, sudden cardiac death and nonfatal infarction in the intervention group compared to the control group. This study also demonstrated that Mediterranean diet can regulate circadian rhythm of cardiac events by its influence of brain function [77]. The Japan Public Health Centre based study [78] showed that increased intake of fish was associated with significant reduction in cardiovascular disease and cardiac mortality. Iso et al. further supported these findings in his study and compared a modest fish intake of once a week or with about 20 g/d [79]. A higher intake of fish was associated with substantially reduced risk of coronary artery disease, primarily nonfatal cardiac events, among middle-aged persons.

Mechanisms

The beneficial effects of the Palaeolithic prudent dietary pattern may be because of low w-6 fatty acids and high content of alpha-linolenic acid (ALA), antioxidants, flavonoids, vitamins amino acids and carotenoids present in the diet [59-64]. Omega-3 fatty acids, such as alpha-linolenic acid (ALA), is rich in mustard oil, walnuts, green leaves, whole grains, and seeds and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are rich in fish and fish oil and can be beneficial in microcirculation, cardiac rhythm and myocardial infarction [59,60].

Table 2. Dietary guidelines and desirable level of risk factors for Indians

Factors	Desirable Values
Energy (k calories/day)	1900-2500
Total Carbohydrate (k calories/day)	65.0
Complex Carbohydrate ((k calories/day)	55.0
Total Fat (k calories/day) (Olive oil may be more)	21.0
Saturated Fatty Acids (k calories/day)	7.0
Polyunsaturated Fatty Acids (k calories/day)	7.0
Polyunsaturated/Saturated Fat Ratio	1.0
n-6/n-3 Fatty Acid Ratio	<5.0
Dietary Cholesterol (mg/day)	100
Whole Grains (wheat, rice, corn, legumes) (g/day)	400-500
Fruit, vegetables and nuts (g/day)	400-500
Salt (g/day)	<6.0
Brisk Walking (km/day)	9.0
Meditation/pranayam (minutes/day)	30.0
Body Mass Index (kg/m^2)	
Range	19.0-23.0
Average	21.0
Waist-Hip Girth Ratio	
Male	<0.90
Female	<0.85
Serum Total Cholesterol (mg/dl) (4.42 mmol/L)	<170
Mild Hypercholesterolemia (mg/dl) (4.42-5.20 mmol/L)	170-200
Hypercholesterolemia (mg/dl) (>5.20 mmol/L)	>200
Low Density Lipoprotein Cholesterol (mg/dl) (2.32 mmol/L)	<90
Borderline High (mg/dl) (2.32-2.84 mmol/L)	90-110
High (mg/dl) (2.84 mmol/L)	>110
Triglycerides (mg/dl) (1.7 mmol/L)	<150
High Density Lipoprotein Cholesterol (mg/dl) (0.9 mmol/L)	>40 men, >50women
Blood Pressure (mmHg), MESOR BP via AMBP.	<125/80
Drug therapy in view of high risk of diabetes and CAD.	Amblodipine, ACE-I , receptor blockers and new beta-blockers? Fish oil, aspirin, flavanols, statins, fibrates.

These nutrients are known to decrease inflammation, insulin resistance and blood lipids in several studies [59,60,80]. Esposito et al (80) randomized 180 patients (99 men, 81 women) with metabolic syndrome to a Mediterranean style diet, characterized with whole grains, vegetables, fruits, nuts and olive oil vs. a cardiac-prudent diet with fat intake <30%. After a follow up of 2 years, subjects in the intervention diet showed greater weight loss, had lower C-reactive protein, and proinflammatory cytokine levels, had less insulin resistance, as well as lower total cholesterol and triglycerides and higher HDL cholesterol. The prevalence of metabolic syndrome was reduced to one half. Since inflammation, hyperlipidaemia, hyperglycaemia, free radical stress and insulin resistance are basic mechanisms responsible for CVDs and other chronic diseases, it poses the possibility that a Mediterranean diet can protect against these problems. A recent study showed that exogenous plant MIR168a specifically targets mammalian LDLRAP1 which is evidence for cross-kingdom regulation by microRNA in humans which is a further proof regarding the mechanism of diet in the prevention of NCDs [81]. The role of physical inactivity in the pathogenesis and of moderate physical activity in the prevention of CVDs and other chronic diseases is well established [28].

Recent guidelines and recommendations have emphasized the role of prevention, early diagnosis and treatment through protocol-based care as well as *via* targeted healthcare interventions not only to mitigate the negative impact of obesity, but also to more precisely measure and closely monitor high blood pressure and hypertension as established risk factors for most non-communicable diseases and CVD, in particular [91-95].

In brief, the Mediterranean style diet or designer foods (400-500g/day) substitution for western pro-atherogenic foods (Table 2), in conjunction with moderate physical activity and cessation of tobacco, may be protective against CVDs and deaths and disability due to CVD and other chronic diseases in most of the low and high middle income and high income populations of the world who are highly susceptible to NCDs.

Acknowledgments

IS has been supported by the Global Platform Research Leader Scheme. Sincere thanks are given to the International College of Nutrition, International College of Cardiology and the Tsim Tsoum Institute for providing logistic support to write this article.

Financial disclosures: None

Conflicts of interest: None has been declared by any of the authors.

Disclaimer: This statement is an opinion expressed by the authors and does not necessarily express the position of the institutions they are affiliated with.

References

[1] Singh RB, Hristova K, Muthusamy VV, Basu TK, Rastogi SS, Toda E, Takahashi T, Fedacko J, Pella D, Wilson DW, De Meester F, Mondal R, Ishaq M, Mohideen MR. The adverse effects of wealth on cardiovascular health: A scientific Statement of the International College of Cardiology. *Cardiol Angiol* 2013;1: 9-22.

[2] Singh RB and Indian Consensus Group. Prevalence and prevention of hypertension, diabetes mellitus, and coronary artery disease in India. A scientific statement of the Indian Society of Hypertension, International College of Nutrition and International College of Cardiology. *World Heart J* 2010; 2: 31-44.

[3] Singh RB, Otsuka K, Shastun SA, Chibisov S, Agarval R. Blood pressure variability and risk of cardiovascular complications. *World Heart* J 2012; 4: 105-108.

[4] Singh RB, De Meester F, Wilczynszka A, Takahashi T, Juneja L, Watson RR. Can a changed food industry prevent cardiovascular diseases? *World Heart J* 2013; 5:1-8.

[5] World Health Organization. The 2008-2013 Action Plan for the Global Strategy for the Prevention and Control of Noncommunicable Diseases (www.who.int/nmh/ Actionplan-PC-NCD-2008.pdf, accessed Jan 2014).

[6] UN. Prevention and control of non-communicable disease. New York: United Nations, 2010. (http://www.un.org/ga/search/view_doc.asp?symbol=A %2F64%2FL.52&Submit=Search&Lang=E, accessed Jan 2014).

[7] Indian Consensus Group: Indian consensus for prevention of hypertension and coronary artery

diseases: a joint scientific statement of Indian Society of Hypertension and International College of Nutrition. *J. Nutr. Environ. Med.* 1996, 6: 309-318.

[8] Singh RB, Mori Hideki, Chen Junshi, Mendis S, Moshiri M, Shoumin Z, Kim SH, Faruqui AMA. Recommendations for the prevention of coronary artery disease in Asians: a scientific statement of the International College of Nutrition. *J. Cardiovas. Risk* 1996; 3: 489-494.

[9] Singh RB, Rastogi SS, Rao PV, Das S, Madhu SV, Das AK, Sahay BK, Fuss SM, Begum R, Sainani GS, Shah NA. Diet and lifestyle guidelines and desirable levels of risk factors for the prevention of diabetes and its risk factors in Indians: A scientific statement of the International College of Nutrition. *J Cardiovasc Risk* 1997; 4: 201-08.

[10] Samb B, Desai N, Mendis S, et al. Prevention and management of chronic disease: a litmus test for health-systems strengthening in low-income and middle-income countries. *Lancet* 2010; 376: 1689–98.

[11] Clark H. NCDs: a challenge to sustainable human development. Lancet 2013; 381: 510-511.

[12] *Editorial.* Wealth but not health in USA. Lancet 2013;381:177. (doi:10.1016/S0140-6736(13)60069-0).

[13] The "Heart Disease and Stroke Statistics - 2014 Update" *Circulation.* 2014; 129: e28-e292 Published online before print December 18, 2013, doi: 10.1161/ 01.cir.0000441139.02102.80

[14] Singh RB, Kumar A, Neki NS, Pella D, Rastogi SS, Basu TK, Otsuka K, De Meester F, Wilson DW. Acharya SN, Juneja L, Takahashi T. Diet and Lifestyle Guidelines and Desirable Levels of Risk Factors for Prevention of Cardiovascular Disease and Diabetes among Elderly Subjects. A Revised Scientific Statement of the International College of Cardiology and International College of Nutrition-2011, *World Heart J* 2011; 3: 305-320.

[15] Singh RB, Anjum B, Takahashi T, Martyrosyan DM, Pella D, De Meester F, Wilson DW, Beehari SSD, Keim M, Shastun S. Poverty is not the absolute cause of deaths due to non-communicable diseases NCDs. *World Heart J* 2012; 4: 221-236.

[16] Moodie R, Stuckler D, Monteiro C, Sheron N, Bruce Neal, Thaksaphon Thamarangsi , Lincoln P, Casswel S on behalf of the *Lancet* NCD Group. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. *The Lancet* 2013 (doi:10.1016/S0140-6736(12)62089).

[17] Singh RB, Takahashi T, Nakaoka T, Otsuka K, Toda E, Shin HH, Kyu Lee M, Beeharry V, Hristova K, Fedacko J, Pella D, De Meester F, Wilson DW, Juneja LR. Nutrition in transition from *Homo sapiens* to *Homo economicus. The Open Nutra J* 2013;6:6-17.

[18] Taylor AW; Price K; Fullerton S. A survey to assist in targeting the adults who undertake risky behaviours, know their health behaviours are not optimal and who acknowledge being worried about their health. *BMC Public Health* 2013; 13, 120

[19] NCD Alliance. Proposed outcome statement for the UN High-Level Meeting on Non-Communicable Diseases.(http://www.ncd alliance. org/sites/ default/files/files/NCD Alliance Proposed Outcomes Document for the UN High-Level Summit.pdf, accessed Jan 2014).

[20] Beaglehole R, Bonita R, Alleyne G, Horton R, Li L, Lincoln P, Mbanya JC, Mckee M, Moodie R, Nishtar S, Piot P, Reddy KS, Stuckler D, for the Lancet NCD Group. UN High-Level Meeting on Non-Communicable Diseases: addressing four questions. *Lancet* 2011; 378:449-455.

[21] Rosenbaum L and Lamas D. Global Health: Facing a "Slow-Motion Disaster" -- The UN Meeting on Noncommunicable Diseases. *N Engl J Med* 2011; 365:2345-2348.

[22] Singh RB, De Meester F, Pella D, Basu TK, Watson R. Globalization of dietary wild foods protects against cardiovascular disease and all cause mortalities? A Scientific statement from the International College of Cardiology, Columbus Paradigm Institute and the International College of Nutrition. *The Open Nutraceuticals Journal*, 2009, 2, 42-45.

[23] WHO. Mortality and burden of disease estimates for WHO Member States in 2008. Geneva: *World Health Organization,* 2010.

[24] Singh RB, Singh V, Kulshrestha SK, Singh S, Gupta P, Kumar R, Krishna A, Srivastava SSL, Gupta SB, Pella D, Cornelissen G. Social class and all-cause mortality in the urban population of north India. *Acta Cardiol* 2005, 60:611-617.

[25] Singh RB, Fedacko J, Vargova V, Kumar A, Mohan A, Pella D, De Meester F, Wilson DW. Singh's verbal autopsy questionnaire for assessment of causes of death, social autopsy, tobacco autopsy, and dietary autopsy based on medical records and interview. *Acta Cardiol* 2011;66:471-481.

[26] *WHO.* Global Status Report on non-communicable diseases 2010. Geneva: World Health Organization, 2011.

[27] Singh RB, Pella D, Mechirova V, Kartikey K, De Meester F, Tomar RS, Begom R, Mehta A, Gupta SB, De AK, Neki NS, Haque M, Naysey J, Singh S, Thakur AS. Rastogi SS, Singh K, Krishna A. Prevalence of obesity, physical inactivity and undernutrition, a triple burden of diseases during transition in a developing economy. The Five City Study Group. *Acta Cardiol* 2007, 62: 119-127.

[28] World Health Organization. Diet and Physical Activity: a public Health Priority. World Health Organization (http://www.who.int/dietphysicalactivity/en/, accessed Jan 2014).

[29] World Health Organization. WHO frame work convention on tobacco control. (http://www.who.int/ fctc/en/index.html, accessed Jan 2014)

[30] Mwatsama M. Raising the political priority of chronic diseases on the global health and development agenda. London: *National Heart Forum*, 2010.

[31] Singh RB, Fedacko J, Pella D, Macejova Z, Ghosh S, Beegom R, Tumbi ZA, Haque M, Vajpeyee SK, De Meester F, Sergey C, Agarval R, Muthusamy VV, Gupta AA, and the Five City Study Group. Prevalence and risk factors of pre-hypertension and hypertension in five Indian cities. *Acta Cardiol* 2011; 66: 29-37.

[32] Singh RB, Suh IL, Singh VP, Chaithiraphan S, Laothavorn P, Sy RG, Babilonia NA, Rahman ARA, Sheikh S, Tomlinson B and Sarraf-Zadigan N. Hypertension and stroke in Asia: prevalence, control and strategies in developing countries for prevention. *J Human Hyper* 2000,14:749-763.

[33] Huang Y, DiDonato JA, Levison BS, et al. An abundant dysfunctional apolipoprotein A1 in human atheroma. *Nat Med* 2014; published online December 26, 2014. DOI:10.1038/nm.3459.

[34] Oberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. *Lancet* 2011; 377: 139–46.

[35] Finucane MM, Stevens GA, Cowan MJ, et al, on behalf of the Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *Lancet* 2011; 377: 557–67.

[36] Danaei G, Finucane MM, Lin JK, et al, on behalf of the Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Pressure). National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5·4 million participants. *Lancet* 2011; 377: 568–77.

[37] 2013 AHA/ACC guidelines on lifestyle management to reduce cardiovascular risk : A report of the American College of cardiology and American Heart Association task force on practice guidelines. Circulation published online November 12, 2013,htpp://circ.ahajournals.org, Jan 12,2014

[38] Farzadfar F, Finucane MM, Danael G, et al, on behalf of the Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Cholesterol). National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3·0 million participants. *Lancet* 2011; 367: 578–86.

[39] Lee PT, Henderson M, Patel V. A UN summit on global mental health. *Lancet* 2010; 376: 516.

[40] Manderbacka K, Sund R, Koski S, Keskimäki I, Elovainio M. Diabetes and depression? Secular trends in the use of antidepressants among persons with diabetes in Finland in 1997–2007. *Pharmacoepidemiol Drug Saf* 2011.20: 338–43.

[41] Stuckler D. Population causes and consequences of leading chronic diseases: a comparative analysis of prevailing explanations. Milbank Q 2008; 86: 273–326.

[42] Abegunde DO, Mathers CD, Adam T, Ortegon M, Strong K. The burden and costs of chronic diseases in low-income and middle-income countries. *Lancet* 2007; 370: 1929–38.

[43] James III AE, Gellad WF, Primack, BA. Implications of New Insurance Coverage for Access to Care, Cost-Sharing, and Reimbursement. *JAMA*. 2014;311(3):241 doi:10.1001/ jama. 2013.283150

[44] Stuckler D, Basu S, McKee M. Drivers of inequality in Millennium Development Goal progress: a statistical analysis. *PLoS Med* 2010;7: e1000241.

[45] Shi W, Chongsuvivatwong V, Geater A, et al. Effect of household and village characteristics on financial catastrophe and impoverishment due to health care spending in western and centralrural China: a multilevel analysis. *Health Res Policy Syst* 2011; 9: 16.

[46] NCD Alliance. Non-communicable diseases. A priority for women's health and development. 2010 (http://www.idf.org/webdata/docs/Women-and-NCDs.pdf, accessed Jan 2014).

[47] Dherani M, Pope D, Mascarenhas M, Smith KR, Weber M, Bruce N. Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. *Bull World Health Organ*. 2008; 86: 390–398C.

[48] Egeland GM, Meltzer SJ. Following in mother's footsteps? Mother-daughter risks for insulin resistance and cardiovascular disease 15 years after gestational diabetes. *Diabet Med* 2010; 27: 257–65.

[49] Creswell J, Raviglione M, Ottmani S, et al. Tuberculosis and noncommunicable diseases: neglected links and missed opportunities. *Eur Respir J* 2011; 37: 1269–82.

[50] Blanco F, San Román J, Vispo E, et al. Management of metabolic complications and cardiovascular risk in HIV-infected patients. *AIDS Rev* 2010; 12: 231–41.

[51] Woodcock J, Edwards P, Tonne C, et al. Public health benefits of strategies to reduce greenhouse-gas emissions: urban land transport. *Lancet* 2009; 374: 1930–43.

[52] Burki TK. Burning issues: tackling indoor air pollution. *Lancet* 2011:377: 1559–60.

[53] Azaria P, Chisholm D, Mathers C, Ezzati M, Beaglehole R. Chronic disease prevention: health effects and financial costs of strategies to reduce salt

intake and control tobacco use. *Lancet* 2007; 370: 2044–53.

[54] Lim SS, Gaziano TA, Gakidou E, et al. Prevention of cardiovascular disease in high-risk individuals in low-income and middle-income countries: health effects and costs. *Lancet* 2007; 370: 2054–62.

[55] Bissell K, Fraser T, Chen-Yuan C, Enarson DA. Smoking cessation and smoke free environments for tuberculosis patients. *Paris: International Union against Tuberculosis and Lung Disease*, 2010.

[56] Cecchini M, Sassi F, Lauer J, Lee YY, Guajardo-Barron V, Chisholm D. Tackling unhealthy diets, physical inactivity, and obesity: health effects and cost-effectiveness. *Lancet* 2010; 376: 1775–84.

[57] Donnelly J. How did Sierra Leone provide free health care? *Lancet* 2011; 377: 1393–96.

[58] Piot P, Ebrahim S. Prevention and control of chronic diseases. *BMJ* 2010; 341: c4865.

[59] Toda E, Toru T, Singh RB, Alam SE, De Meester F, Wilczynska A, Wilson DW. *Palaeolithic style diet and coronary artery disease: The tissue is the issue*. 2012; 2:183-193.

[60] De Meester F. Wild-type land based foods in health promotion and disease prevention: the LDL-CC:HDL-CC model. In *Wild Type Foods in Health Promotion and Disease Prevention,* editors Fabien DeMeester and RR Watson, Humana Press, NJ 2008,p 3-20.

[61] Halberg F , Cornélissen G, Otsuka K, Siegelova J, Fišer B , Dušek J, Homolka J, Salvador S Peña DL, Singh RB and the BIOCOS project. Extended consensus on need and means to detect vascular variability disorders (VVDs) and vascular variability syndromes (VVSs). *Intl. J. of Geronto-Geriatrics*, 2008;11: 119-146.

[62] Wilson DW, Hungin APS, Howse JH, De Meester F, Singh RB, Wilczynska A, Cornelissen G, Halberg F. Role of hormones and neuropeptides in IBS and other gastrointestinal disorders: Understanding Variability and Chrononutrition. *The Open Nutra Jour* 2011;4:213-225.

[63] Kastorini CM, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The effect of Mediterranean diet on metabolic syndrome and its components. *JACC* 2011;57:1299-313.

[64] Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence about benefits of adherence to Mediterranean diet on health: an updated systematic review with meta-analysis. *Am J Clin Nutr* 2010; 92:1189-1196.

[65] McCall DO, McGartland CP, McKinley MC, Patterson CP, Sharpe P, McCance CR, Young IS, Woodside JV. Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. *Circulation.* 2009;119: 2153-2160.

[66] He FJ, Nowson CA, Lucas M, MacGregor GA. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *J Hum Hypertens* 2007; 21: 717-28.

[67] Gardener H, Wright CB, Gu Y, Demmer RT, Boden-Albala D, Alkind MSV, Sacco RL, Scarmeas N. Mediterranean style diet and risk of ischemic stroke, myocardial infarction and vascular death : the Northern Manhattan Study. *Am J ClinNutr* 2011;94:1458-64.

[68] Heidemann C, Schulze MB, Franco OH, et al. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. *Circulation* 2008; 118: 230-7.

[69] Trichopolou A, Bamia C, Trichopolous D. Anatomy of health effects of Mediterranean diet. Greek EPIC prospective cohort study. *BMJ* 2009; 338: b2337

[70] Iqbal R, Anand S,Ounpuu S, et al. Dietary patterns and the risk of acute myocardial infarction in 52 countries. *Circulation* 2008; 118: 1929-37.

[71] Singh RB, Rastogi SS, Verma R, Bolaki L, Singh R, Ghosh S. An Indian experiment with nutritional modulation in acute myocardial infarction. *Am J Cardiol.* 1992; 69 : 879-85.

[72] Singh RB, Rastogi SS, Verma R, Laxmi B, Singh R, Ghosh S, Niaz MA. Randomized, controlled trial of cardioprotective diet in patients with acute myocardial infarction: results of one year follow up. *BMJ* 1992;304:1015-19.

[73] Singh RB, Fedacko J, Vargova V, Niaz MA, Rastogi SS, Ghosh S. Effect of low W-6/W-3 ratio fatty acid Palaeolithic style diet in patients with acute coronary syndromes. A randomized, single blind, controlled trial. *World Heart J* 2012;3:71-84.

[74] Burr ML, Fehily AM, Gilbert JF. Effects of changes in fat, fish and fibre intakes on death and myocardial infarction: Diet and ReinfarctionTrial (DART). *Lancet* 1989,ii:757-761.

[75] de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet. 1994, 343(8911):1454-9. *Erratum in: Lancet* 1995,345 (8951):738.

[76] Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, Manor O, Pella D, Berry EM. Effect of an Indo-Mediterranean diet on progression of coronary disease in high risk patients: a randomized single blind trial. *Lancet* 2002, 360:1455-1461.

[77] Gal D, Pella D and Singh RB. The effect of an alpha-linolenic acid rich diet on circadian rhythm of cardiac events. *World Heart J* 2008;1:49-56.

[78] Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S, for the JPHC Study Group. Intake of Fish and n3 Fatty Acids and Risk of Coronary Heart Disease Among Japanese: The Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation* 2006;113 195-202. 64.

[79] Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004; 292:1440-1446.

[80] Iso H, Kobayashi M, Ishihara J, Sasaki J, Okada K, Kita Y, Kokubo Y, Tsugane A for the JPHC Study Group. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: The Japan Public Health Center-Based (JPHC) *Study Cohort I. Circulation* 2006;113 195-202.

[81] Singh RB, Beegom R, Mehta AS et al, Social class and coronary risk factors and undernutrition: a double burden of diseases among women in five Indian cities. *Int J Cardiol*, 1999,353:154-65.

[82] Halberg F, Cornelissen G, Otsuka K, Siegelova J, Fiser B, Dusek J, Homolka P, Sanchez de la Pena S, Singh RB and the BIOCOS Project. Extended consensus on need and means to detect vascular variability disorders (VVDs) and vascular variability syndromes (VVSs). *Intl. J. Geronto-Geriatrics* 2008, 11 (14) 119-146.

[83] Gumarova LZ et al. Duration of ABPM as an important prerequisite for a reliable diagnosis of vascular variability disorders (VVDs). *Vestnik RUDN* 2013, 1, 27-33.

[84] Keogh C, Wallace E, Dillon C, Dimitrov BD, Fahey TP. Validation of the CHADS2 clinical prediction rule to predict ischaemic stroke. A systematic review and meta-analysis. *Thromb Haemost* 2011; 106(3):528-38.

[85] Galvin R, Geraghty C, Motterlini N, Dimitrov BD, Fahey T. Prognostic value of the ABCD2 clinical prediction rule: a systematic review and meta-analysis. *Family Practice* 2011; 28(4): 366-376.

[86] Dimitrov, BD, Motterlini, N, Fahey, T. New simplified approach to the pooled analysis of calibration of clinical prediction rules for systematic reviews of validation studies. 32nd Conference on Applied Statistics in Ireland, Dundalk, Ireland, 16-18 May 2012, 56.

[87] Halberg F, Powell D, Otsuka K, Watanabe Y, Beaty LA, Rosch P, Czaplicki J, Hillman D, Schwartzkopff O, Cornelissen G. Diagnosing vascular variability anomalies, not only MESOR-hypertension. *Am J Physiol Heart Circ Physiol* 2013; 305: H279-H294.

[88] Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornelissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. *Biomed & Pharmacother* 2001; 55 (Suppl 1): 125s-132s.

[89] Watanabe Y, Halberg F, Otsuka K, Cornelissen G. Toward a personalized chronotherapy of high blood pressure and a circadian overswing. *Clin Exp Hypertens* 2013; 35 (4): 257-266.

[90] Cornelissen G, Halberg J, Halberg F, Sanchez de la Pena S, Nelson W, Schwartzkopff O, Stoynev A, Haus E. Schedule shifts, cancer and longevity: good, bad or indifferent? *J Experimental Therapeutics Oncol* 2008; 7 (4): 263-274.

[91] Eggleston EM, Finkelstein JN. Finding the role of health care in population health. JAMA 2014; Early online view (doi:10.1001/jama.2014.163)

[92] Jensen MD, Ryan DH. New obesity guidelines, promise and potential. *JAMA* 2014,311: 23-24.

[93] Frieden TR, Sallyann M. King C, Wright JS. Protocol-Based Treatment of Hypertension: A Critical Step on the Pathway to Progress. *JAMA*. 2014; 311(1):21 (doi:10.1001/jama.2013.282615)

[94] Mancia G, Fagard R, Narkiwicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the task force for the management of hypertension of the European Society of Hypertension(ESH) and the European Society of Cardiology (ESC). *Eur Heart J* 2013; 34(28):2159-2219.

[95] James PA, Oparil S, Carter BL, Cushman WC et al. Evidence based guideline for the management of high blood pressure in adults: Report from the panel members appointed to the 8th Joint National Committee (JNC 8). *JAMA* 2013, Early online view (doi:10.1001/jama. 2013.284427).

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Validity of Verbal Autopsy Questionnaire for Assessment of Causes of Death among Patients with Hypertension in Bangladesh

Ratindra Nath Mondal[*1], Md. Ashraful Haque[2], Shah Md. Sarwer Jahan[2], Abul Kalam Azad[2], Md. Mahfuzer Rahman[2], Moni Rani[2], B. D. Bidhu[2], A.K.M. Shaeheduzzaman[2], Md. Zakir Hossain[2], Md. Kumruzzaman Sarker[2], Amaresh Chandra Shaha[2], Ram B. Singh[3], Md. Shafiul Alam[2], Md. Noor Islam[2], and Devendra Nath Sarker[2]

[1]Hypertension and Research Center
[2]Rangpur Medical College
[3]Halberg Hospital and Research Institute, Moradabad, India

Abstract

Background: Hypertension has become as an important cause of premature mortality and morbidity due to its major etiologic role in the development of coronary artery disease (CAD), stroke and renal failure. The present study aims to determine the validity of verbal autopsy questionnaire in our setting to find out the causes of death among known hypertensive patients.

Subjects Design and Methods: Of 200 decedents approached from July 2013 to December 2013, relatives of only 118 responded for this study. All the families of these victims were contacted individually to find out the cause of deaths by verbal autopsy questionnaire. Every effort was made to find out the closest relative of the victim, who was present at the time of death of the deceased. A trained health worker completed the verbal autopsy questionnaire by asking leading questions in a structured interview. Clinical manifestations of the primary cause of death were collected by the principal investigator by asking leading questions over telephone. Further clinical data and investigations were collected from available records of our center and the board of experts examined all the available clinical data and finalized the cause of death of the victim. In case of available death certificate, mentioned cause of death was reassessed. Students t test and chi square test were used for comparison of the data.

Results: Of 118 victims assessed; mean age at the time of death was 62.40 (SD 62.2years). Male victims were greater than females 72.9 vs 27.1%. Majority (71.2%) of the victims died due to hypertension related complications; 33.3% due to stroke, 20.3% CAD and 17.8% chronic kidney disease. Rest 28.82% of the victims died due to other causes unrelated to hypertension, e.g. 10.2% due to malignancy and in 13.6%, the cause could not be determined. Only 15.5% of the victims who died due to hypertension related complications were under regular follow up. The mean follow up blood pressure were 155.54/90.01 (uncontrolled), and mean duration of hypertension at the time of death was 5.77 years. Majority of the subjects were either illiterate or only literate having lower income farmers and house wives.

Conclusion: Verbal autopsy accurately determined the causes of deaths in known patients of hypertension. CAD,

[*] **Correspondence:** Dr. Ratindra Nath Mondal, Associate Professor of Medicine, Hypertension and Research center, Rangpur, Rangpur Community Medical College; Rangpur, Bangladesh, Email; dr.ratinmondal@gmail.com.

stroke and chronic kidney disease were common causes of deaths. Majority of the victims were from lower social classes. Ignorance and poor literacy rate appear to be the important risk factors for premature death among these patients.

Keywords*:* Stroke, heart attack, hypertension, education, social class

Introduction

NCDs were estimated to have contributed to almost 60% of deaths in the world and among them about 80% occurred in the developing countries [1] Of total number of deaths, 51% of deaths in Bangladesh occur due to NCDs and other chronic health conditions [2] High blood pressure is an important preventable cause of mortality, and accounts for a substantial portion of global burden of disease. [3,4] People with hypertension have higher rates of mortality than people without hypertension, which is mainly due to vascular diseases [5,6] Among the NCDs, hypertension is the major risk factor for developing coronary artery disease (CAD), stroke, heart and renal failures and peripheral vascular diseases [7] Hypertension has been reported to be responsible for 57 per cent of all stroke deaths and 24 per cent of all cardiovascular deaths in East Asians [8] A marked increase in prevalence of hypertension (from 11.3% to 17.9%) was observed in Bangladesh from 1999 to 2010. [9,10] In Rangpur division (Northern part) of Bangladesh prevalence of hypertension and pre-hypertension is 33.3% and 29.9% respectively.[11] Because of the high prevalence of this condition and the increased morbidity and mortality associated with this condition, the economic cost of hypertensive disease was estimated at $76.6 billion in 2010. [12] Many developing countries with the highest burden of poverty and disease continue to lack routine, representative and high quality information on the causes of death.[13,14] This is crucial for health and development policies, health programs; program monitoring and evaluation purposes [15]. In this regard, mortality surveillance systems and demographic surveillance sites using validated verbal autopsy procedures appear to be a cost-effective alternative method for ascertaining cause of death and sustainable medium-term solutions [16,17]. As a result, verbal autopsy method has been an epidemiological tool for the last few decades to estimate cause specific mortality of child, maternal and adult deaths. [16, 18]

Causes of deaths are less commonly known in developing countries, although hospital records indicate a rapid increase in mortality and morbidity due to NCDs. [19]. Due to religious considerations, autopsy is not possible in many countries, therefore WHO experts and the International College of Nutrition, International College of Cardiology and the Tim Tom Institute have developed a verbal autopsy questionnaire to find out the causes of deaths in these countries. [20, 21]. Verbal autopsy is a method by which we interview the relatives or health professionals to find out the signs, symptoms, attributes of social class and other characteristics experienced by the deceased before their death and the circumstances surrounding their death [22]. It is based on the assumption that most of the causes of deaths can be distinguished by their signs and symptoms and that these can be accurately recognized, recalled and reported by lay respondents [23]. Singh et al has shown that causes of deaths can be accurately assessed by a modified version of verbal autopsy questionnaire (WHO), based on medical records and interview of the family members [24]. In the present study, we report the estimates for the causes of death among known hypertensives, based on modified Singh's verbal autopsy questionnaire[24] which is completed with the help of spouse and available medical records of the victim at our center.

Methodology

Populations and Methods

Hypertension and Research Center, Rangpur was established on 14th November, 2008, serves only those patients with hypertension, to generate awareness of hypertension in the population of Bangladesh. Approximately, 12 thousand patients have been registered in this center till 2013. If any patient does not come for follow up for 3 months after the scheduled follow up visit, he/she is declared as drop out from follow up and a telephone call is given to know the cause of drop out. Our study was approved by the ethic committee of our center and we

took informed consent from the family members who were interviewed by our health worker.

We have been successful in tracing 200 patients (by approximately 5000 telephone calls?), who died between November, 2008 to December, 2013. Of these 200 decedents, only 118 could be included in this study depending upon cooperation from the family members of the victim. Rest 82 victims could not be contacted due to non-cooperation and our failure in motivation of the family members of the victim. At least three calls were made before any subject or family was declared a non-contact or non-replier; one in the morning, one in the evening, around 17.00 hrs, and the last one at the weekend. The family members preferably spouse of these 118 victims, were contacted individually to find out the causes of deaths by verbal autopsy questionnaire.

Verbal Autopsy Procedure

Our health worker visited the household of the decedent, between July 2013 to January 2014, to find the closest relative son/daughter/wife/husband of the victim, who was present besides the deceased before the time of death. The family members, for motivation, were given to understand that inquiry about the cause of death aims to prevent illness and death in your other family members which can be done by changing the health bahaviour. The health worker was trained in understanding the questionnaire and in data collection, during the structured interview (Appendix 1). The interview consisted of a modified Singh,s verbal autopsy questionnaire [24] to record the socio-demographic and clinical characteristics of the deceased as well as clinical manifestations at the time of illness and cause of death. Symptoms and clinical data before the time of death were confirmed over telephone, and recorded by the principal investigator. Finally any available medical records or death certificate were collected from each of the family. The medical records, follow up status, modifiable risk factors of hypertension, investigation reports, know target organ damage and mean follow up blood pressure were collected from records system of Hypertension and Research Center, Rangpur to find the cause of death. A board consisting of internist, cardiologist, neurologist, nephrologist, endocrinologist, pulmonologist was appointed to review all the available clinical data to finalize the cause of death. In case of available death certificate, the mentioned primary cause of death was also assessed in the light of available evidence in our record system.

Criteria of Diagnosis

Hypertension was diagnosed due to presence of systolic or diastolic blood pressure or both ≥140/90 mm of Hg (according to NICE guideline). Or any individual diagnosed as hypertension and currently taking antihypertensive drugs.

Data Collection

Data regarding attributes of social classes; education of the victim, occupation and family income as well as per capita income were collected by interview of close relative, preferably spouse of the decedents. Medical records of the victims were taken and examined from the register of medical records available at Hypertension and Research Center, Rangpur.

Statistical Analysis

The data were analyzed by SPSS windows version 17.0. The socio-demographic data of the study population were expressed in frequency distribution and their observed difference was tested by one sample‘t’ test and ‘chi square’ test. P value <0.05 was and two tailed t test were considered as statistically significant with the 95% confidence interval.

Results

Of 200 victims, whose families were contacted, 118 family members volunteered for the interview. Mean age of the victims at the time of death were 62.40 years and males were more common than female (72.9% vs 27.1%, P<0.01) (Table 1).

Table 1. Socio-demographic characteristics of the victims. Values are number (%)

Variables	Number (n=118)	Percentage
Mean age (SD)years	62.40 ±12.21	
Mean body weight (Kg/M2)	21.59 Kg/M2	
Sex-Male	86	72.9%*
Female	32	27.1%
Level of education		
Illiterate	16	13.6%
5 or less class	28	23.7%
>5-10 class	40	33.9%
>10-12 class	19	16.1%
Graduate and above	15	12.7%
Occupation		
Housewife	31	26.3%
Agriculture	36	30.5%
Business	15	12.7%
Service	34	28.8%
Retired	1	0.8%
Others	1	0.8%
Monthly income		
<62.5 USD	33	28%
62.5-125 USD	45	38.1%
125-187.5 USD	30	25.4%
>187.5 USD	10	8.5%

*= P<0.01.

Table 2. Causes of death among decedents with hypertension

Cause of death	Male (n=86)		Female (n=32)		Total (n=118)
Stroke	29	33.7%	10	31.3%	33.1 (39)
Coronary artery disease	19	22.1%	5	15.6%	20.3 (24)
Chronic kidney disease	14	16.3%	7	21.9%	17.8 (21)
Malignancy	8	9.3%	4	12.5%	10.2 (12)
Diabetic ketoacidosis	1	1.2%	00	00	2.5 (3)
Post diarrhoeal ARF	00	00	1	3.1%	0.8 (1)
Traffic accident	3	3.5%	00	00	0.8 (1)
Electric shock	00	00	1	3.1%	0.8 (1)
Undetermined	12	14%	4	12.5%	13.6 (16)

ARF= acute renal failure.

Approximately 81.4% of the victims were from rural areas. Majority of the victims (57.6%) were educated from the 5^{th} to 10^{th} class and higher education was least common. The decedents were mainly house wives among females and farmers among males with poverty and ignorance (Table 1).

Only 16.1% (n=19) of the hypertensive patients were coming for regular follow up, 30.5% (n=36) were on irregular follow up and 53.4% (n=60) did not come to any follow up (p value 0.00001558). Among victims who regularly or irregularly came for follow up, blood pressure was controlled only in 20%. Among the controlled hypertensive subjects, causes of death were unrelated to hypertension e g, malignancy, traffic accidents, electric shock etc in 45.46%, where as in uncontrolled hypertensive subjects, these causes were observed only among 25% (P value =0.1336144).

Majority of the victims (71.2%) died due to stroke, CAD and chronic renal failure (Table 2). Rest 15.2 % of the victims died due to non-hypertension related diseases (e.g. malignancy, traffic accidents, electric shock etc). The causes of deaths were undetermined in 13.6%. (Table 2).

Among the three leading cause of death; CAD, Stroke, chronic kidney disease, percentage of death was significantly higher among men than in women victims with stroke (p = 0.00234725) and CAD (p = 0.00426594). (Figure 1)

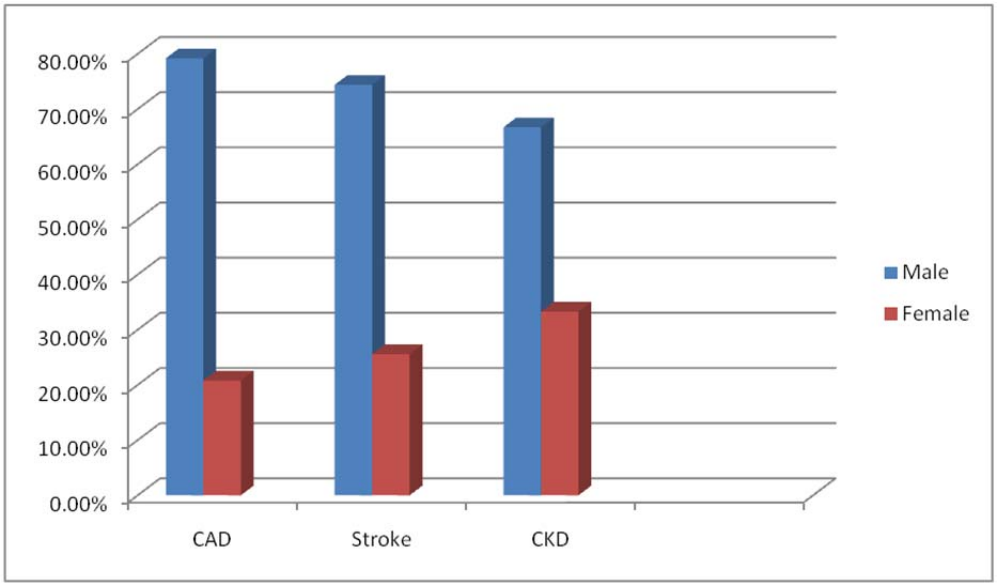


Figure 1. Proportions of deaths due to CAD, stroke and chronic kidney disease among men and women. CAD= Coronary artery disease, CKD= chronic kidney disease.

Discussion

High blood pressure is the leading cause of mortality around the world. Though prevalence, awareness and control rate of hypertension is increasing day by day, there is limited data on causes of death in hypertensive patients from South Asia [1,2]. Verbal autopsy questionnaire has been used to find out the causes of death among hypertensive subjects for the first time in Bangladesh.

This study shows that 71.2% of the victims died due to complications of hypertension; 33.3% due to stroke, 20.3% due to CAD and 17.8% due to chronic renal failure (Table 2). Hua Cui et al have shown causes of death among elderly hypertensive patients in a hospital-based sample of Chinese subjects indicating cardiovascular disease in 45.2%, cerebro-vascular disease in 34.3% and renal failure in 11.9%[25] It is clear that the proportions of deaths from cardiovascular and cerebrovascular disease are higher in this study compared to our study. A possible explanation may be that this study was a hospital based study carried out among the elderly patients in which these problems are more common (>60 years).

Singh et al showed that using the modified verbal autopsy questionnaire allowed to diagnose 23.4% deaths due to heart diseases and 9.8% due to brain diseases, including stroke and inflammatory brain diseases [24]. However, Khademi et al used other verbal autopsy questionnaire based on WHO guidelines, where CAD could be diagnosed only in 10.0% and cerebrovascular disease in 7.8% and rest causes of CV deaths were classified into the "other" causes of death [26]. In this study, only transport accidents were included in the questionnaires, but accidental deaths according to modified questionnaires were much higher (14.0% vs. 2.3%) [24,29]. Renal diseases including acute renal failure and chronic renal failure were the cause of death among 11.2% of victims, which are difficult to

diagnose using the other questionnaire [26]. Among "other" causes of death, comparing modified questionnaire, with WHO questionnaire revealed that there were 37.3 vs. 8.5 % deaths respectively, due to other causes which means that among one third of victims, causes of death could not be classified according to body systems [24-26]. Singh et al observed that using the modified questionnaires, most of the victims could be classified systematically, relatively more accurately, into various causes of death according to body systems [24]. In the present study, we used similar approach, but, in 13.6% victims, we failed to determine the cause of death, due to inadequate history from the relatives which may be due to illiteracy and ignorance. Suicides, AIDs and tuberculosis are possible causes of deaths in this subgroup, because these causes are not disclosed by the relatives due to socio-cultural reasons.

Verbal autopsy questionnaire allowed the health workers to ascertain the clinical presentation of the victim during illness and up to death. These details may or may not be available in the medical records and from the spouse and other family members and doctors of the decedents.

This information on probable cause of death related to each body system was reviewed by the internist physician (RNM) to assess the accurate cause of death among all the victims. In patients with known hypertension, using the modified questionnaires, most of the decedents could be classified systematically in accordance of target organ damage due to hypertension, relatively more accurately, into various causes of death (Table 2).

Our verbal autopsy questionnaire appears to be a valid and reliable supplemental method to assess causes of death in a population where records of death are not yet maintained in a municipal corporation in any developing country. The success of our verbal autopsy may be due to the appropriate modifications made in the WHO and Singh's questionnaires to adapt it to local setting and to known patients of hypertension (24).

Verbal autopsy methodology and questionnaire appear to have many variations depending upon the investigators [14-18, 24-31]. In some of them, ICD coding system for the causes of death may not have been used and hence WHO has recently published instructions to improve In many low resource countries, verbal autopsy has been found to provide more valid causes of death compared to routine death certificates issued by the doctors [14-18,24-31].

In few studies using verbal autopsy for finding out the causes of death among adults, did not find similar results. Yang et al reported that verbal autopsy is not a very precise tool for assessing the leading causes of death among adults and provide only marginal support to this method [29]. It seems that verbal autopsy is a developing method which may be the cause of inconsistency in results in some of the studies [29].

This verbal autopsy method among known patients of hypertension appears to be a promising approach and of gold standard in identifying most precisely the exact cause of death which may be compared with postmortem. However, causes of deaths due to myocarditis, cardiomyopathy, pericarditis, aneurism of aorta and oesophgeal problems in relation to heart attack may be missed,unless records are available. One important weakness in our study is that we could not compare our results with causes of death obtained by death certificate alone verses verbal autopsy combined with medical records by using kappa statistics.

In the verbal autopsy by Singh et al, only one internist physician reviewed the records of all the victims and only when diagnosis of cause was doubtful, a second opinion was taken from another internist physician [24]

In our study a board of super-specialists was available to review the records to finalize the cause of death. It is possible that many people die at home due to lack of resources for hospitalization, although government hospitals provide free service to poor in most of the towns in developing countries.

In future, use of MP3 players to compare audio (observed on computer) with paper record on a 5% population to determine the degree of concordance of the autopsy questionnaire can further improve the verbal autopsy.

In a recent study, non-communicable diseases, sensitivity of the verbal autopsy diagnoses was 69% (CI, 62%–76%), specificity 78% (CI, 71%–85%) and positive predictive value 79% (CI, 73%–85%). Sensitivity was highest for stroke 68% (CI, 49%–87%), and lower for respiratory diseases 24% (CI, 3%–44%). [27] lower socioeconomic status, health

perception and low level of education can also be important.

A few studies has shown monthly income of the hypertensive patients was <5000 BDT (62.5 USD). [11,32] In the present study majority of the hypertensive had monthly income 5000-10000 BDT (62.5-125 USD).

The target organ damage of hypertensive patient in different study [11,33] was found 43.75%-44.5% which is similar to our study (39.83%) Figure 1 in our study, CAD, stroke and chronic renal failure were predominantly found among males, with a high male-female ratio. This may be due to hormonal protection of female against cardiovascular diseases and dependency of the female to male to get health care facilities in developing countries.

In brief, verbal autopsy appears to be an accurate method to find out the causes of death among known patients with hypertension. Majority of the hypertensive patients died due to target organ damage to different body systems; stroke, CAD chronic renal failure due to illiteracy and ignorance. A cohort study with large sample size will be needed to clarify the causes of death in hypertensive patients.

Limitation

Sample size was small and data were collected in some of the patients many days (even up to 4 years) after the death of the victims.

Conflict of interest

There was no conflict of interest.

Acknowledgment

The authors of this study are grateful to Professor M. A. Jalil Chowdhury, Professor Dr. Md. Mujibur Rahman, staff of Hypertension and Research Centre, Rangpur; Department of Medicine, RpMCH, Department of Medicine of Rangpur Community Medical College.

Contributions by the authors

Dr. Ratindra Nath Mondal planned and supervised the study. Dr. Md. Ashraful Haque, Dr. Abul Kalam Azad analysed the data. Dr. Shah Md. Sarwer Jahan, Dr. Md. Mahfuzer Rahman, Dr. Md. Kumruzzaman Sarker, Dr. Moni Rani, AKM Shaeheduzzaman, Md. Shafiul Alam, Devendra Nath Sarker, helped to collect the data. Dr. B. D. Bidhu, Professor Dr. Md. Zakir Hossain, Professor Dr. Amaresh Chandra Shaha, Professor Ram B. Singh, Professor Dr. Md. Noor Islam helped in writing of the manuscript and presentation of the data.

References

[1] World Health Organization. Global status report on non-communicable diseases 2010. WHO Geneva 2011.

[2] Bangladesh Bureau of Statistics (2007) *Statistical Pocketbook of Bangladesh 2007*. Dhaka: Bangladesh Bureau of Statistics. Retrieved 13 May 2009 from http://www.bbs.gov.bd/dataindex/pb_wb_page.pdf.

[3] Ezzati M, Lopez AD, Rodgers A, Vander HS, Murray CJ. Selected major risk factors and global and regional burden of disease. Lancet. 2002; 360: 1347–1360

[4] Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M. The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med. April 28, 2009. doi:10.1371/journal.pmed.1000058. http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1000058.Accessed March 22, 2011.

[5] Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet.* 2002; 360: 1903–1913.

[6] Murakami Y, Hozawa A, Okamura T, Ueshima H. Relation of blood pressure and all-cause mortality in 180 000 Japanese participants: pooled analysis of 13 cohort studies. *Hypertension*. 2008; 51: 1483–1491

[7] World Health Organization. World Health Report 2002: Reducing risks, promoting healthy life. *WHO Geneva* 2003.

[8] Rodgers A, Lawes C, MacMahon S. Reducing the global burden of blood pressure related cardiovascular disease. J *Hypertens* 2000; 18 (Suppl 1):S3-6.

[9] MM Zaman and MA Rouf. Journal of Human *Hypertension* (1999) 13, 547–549.

[10] NCD risk factor survey 2010 report published and disseminated published on 7th August

2011http://www.ban.searo.who.int/EN/Section31_295.htm.

[11] Mondal RN, Haque MA, Jahan SMS, Azad AK, Rahman MM, Rani Moni et al; Prevalence and risk factors of hypertension in Rangpur, Bangladesh; *World Heart J*, 2013;5: 91-100.

[12] Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De SG, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J. Heart disease and stroke statistics: 2010 update: a report from the American Heart Association. *Circulation.* 2010; 121: e46–e215.

[13] Mathers C, Ma Fat D, Inoue M, Rao C, Lopez AD: Counting the dead and what they died of: an assessment of the global status of cause of death data. *Bull World Health Organ* 2005, 83:171-177.

[14] Anker M: The effect of misclassification error on reported cause-specific mortality fractions from verbal autopsy. *Int J Epidemiol* 1997, 26:1090-1096.

[15] Coldham C, Ross D, Quigly M, Segura Z, Chandramohan D: Prospective validation of a stadardized questionnaire for estimating childhood mortality and morbidity due to pneumonia and diarrhea. *Trop Med Int Health* 2000, 5:134-144.

[16] Setel PW, Sankoh O, Rao C, Velkoff VA, Mathers C, Gonghuan Y: Sample registration of vital events with verbal autopsy: a renewed commitment to measuring and monitoring vital statistics. *Bull World Health Organ* 2005, 83:611-617.

[17] Chandramohan D, Setel P, Quigley M: Effects of misclassification of cause of death in verbal autopsy: can it be adjusted? *Int J Epidemiol* 2001, 30:509-514.

[18] Rodriguez L, Reyes H, Tome P, Ridaura C, Flores S, Guiscafre H: Validation of the verbal autopsy method to ascertain acute respiratory infection as cause of death. *Indian J Pediatr* 1998, 65:579-584.

[19] World Health Organization. The World Health Report 2002, Reducing risks, promoting healthy life. *WHO,* Geneva 2002.

[20] Singh RB, Singh S, Singh V, Kulshresth SK, Mechirova V,Pella D. Association of increased mortality with underweight, overweight and obesity among urban descents in North India dying due to various causes. *World Heart J* 2008; 1:341-347.

[21] Singh RB, Singh V, Kulshrestha SK, Singh S, Gupta P,Kumar R, Krishna A, Srivastav SSL, Gupta SB, Pella D, Cornelissen G. Social class and all cause mortality in an urban population of north India. *Acta Cardiol* 2005,60:611-617.

[22] Baiden F, Bawah A, Biai S, Binka F, Boerma T..Setting international standards for verbal autopsy. *Bull World Health Organ* 2007; 85:570–571.

[23] Snow RW, Armstrong JR, Forster D, Winstanley MT, Marsh VM, Newton CR: Childhood deaths in Africa: uses and limitations of verbal autopsies. *Lancet.* 1992, 340:351-355.

[24] Singh RB, Fedacko J, Vargova V., Kumar A., Mohan V., Pella D., De Meester F, Wilson DW. Singh's verbal autopsy questionnaire for assessment of causes of death, social autopsy, tobacco autopsy and dietary autopsy, based on medical records and interview, *Acta Cardiol* 2011;66: 471-481.

[25] 25.Hua Cui, Yixin Hu, Changming Hong, Guoliang Hu, Li Fan; A 15 years study of the causes of death among elderly hypertensive patients in a hospital-based sample of China; *Archives of Gerontology and Geriatrics*; 2012,55: 709-712.

[26] Khademi H, Etemadi A, Kamanger F, Nourats M. Verbal autopsy: reliability and validity estimates for causes of death in the Golestan cohort study in Iran. *PLoS ONE*, 2010; 5:1-

[27] Awoke Misganaw, Damen Haile Mariam, Tekebash Araya and Aderaw Aneneh; Validity of verbal autopsy method to determine causes of death among adults in the urban setting of Ethiopia; *BMC Medical Research Methodology* 2012, 12:130 doi:10.1186/1471-2288-12-130.6.

[28] Soleman N, Chandramohan D, Shibuya K. Verbal autopsy: current practices and challenges. *Bull World Health Organ* 2006; 84: 239–245.

[29] Yang G, Rao C, Ma J, Wang L, Wan X. Validation of verbal autopsy procedures for adult deaths in China. *Int J Epidemiol* 2006; 35: 741–748.

[30] Jha P, Gajalakshami V, Gupta PC, Kumar R,Mony P, Dhingra N, Peto R, RGI-CGHR Study collaborators. Prospective study of one million deaths in India: rationally, design and validation results. *PLoS Medicine* 2006; 3:191-201.

[31] Gajalakshmi V, Peto R. Verbal autopsy of 80,000 adult deaths in Tamilnadu, South India. *BMC Public Health* 2004; 4: 47.

[32] Mondal RN, Haque MA, Jahan SMS, Azad AK, Rahman MM, Rani Moni,Saha AC. NurslamM,Hossain MJ. Frequency of Causes of Dropout among Patients with Hypertension; *World Heart Journal* 2012;4: 293-300.

[33] Athanasia Papazafiropoulou, Efstathios Skliros, Alexios Sotiropoulos, Christos Papafragos, Aristofanis Gikas, Ourania Apostolou, Hariklia Kaliora and Charalambos Tountas. Prevalence of target organ damage in hypertensive subjects attending primary care: C.V.P.C. study; *BMC Family Practice* 2011, 12:75.

Appendix. I

HYPERTENSION AND RESEARCH CENTER, Rangpur, Bangladesh.
VERBAL AUTOPSY QUESTIONNAIRE Modified from Singh's Verbal Autopsy Questionnaire
INTERVIEW.

Name and post of expert filling the Questionnaire……Telephone…….
History given by: Spouse / Father / Mother / Sister / Son / Daughter / In-laws (tick)
Education of person……
Date……..Place……Time of Interview…….
Unique ID……..

GENERAL DATA:

Name of Victim……..……Surname…… Sex……Age…
Address……
……Tel No……
1. Religion: (a) Hindu (b) Moslem (c) Christian (d) Sikh
2. Final education of victim……Occupation of victim……Family Type……
3. The date…… Place ……. and Time of Death……..
4. Physically matching subject with victim/Name, address……

CLINICAL DATA OF VICTIM. (By Medical Records)

5. Family history of diabetes, hypertension, cholesterol, heart attack, stroke, cancer, Bronchitis, asthma, obesity, suicide, HIV, depression, psychosis, alcohol intake, tobacco.
6. Any past history of above, illnesses (Name)
7. Height ……cm ….Weight ….Kg, BMI… (Kg/M 2)
8. Waist circumference…… cm, maximum hip circumference……WHR……
9. Blood pressure…….. mmHg, Grey hair/Cataract/Wrinkles. (Tick)
10. History of surgery……
11. Chest Pain. ……..……..
12.CLINICAL MANIFESTATIONS.(Ask leading questions to know the symptoms)

Headache, vomiting, fever, loss of consciousness, convulsions, breathlessness, chest pain, sweating, palpitation, heaviness in chest, nausea, vomiting, coughing, burning in urine, decrease in urine, h/o depression, diabetes mellitus, heart attack, sudden cardiac death, paralysis, trauma etc., loose motions with persistent vomiting, persistent headache with fever and vomiting, persistent cough with high fever;

Yellow eyes, bleeding from any part of body.

Table. Causes of deaths reported based on available records and verbal autopsy (modified from WHO)
(Ask leading questions to know the system involved)

Cause of death based on Death Certificate	interview of Spouse/relative/ Doctor Final cause
1. Circulatory diseases; Coronary artery disease, Sudden cardiac death, Diabetes, Vascular disease, Valvular heart disease, Inflammatory cardiac disease.	
2. Nervous system diseases; Cerebrovascular disease, Meningitis, encephalitis.	
3. Malignant neoplasm; Lung, oral, liver, stomach, breast, uterus, cervix, ovary.	
4. Injury- accidents; Unintentional, road accidents, poisonings, fires, falls, drowning.	
5. Renal diseases. Acute renal failure, chronic renal failure.	
6. Pulmonary diseases; Chronic bronchitis, asthma, tuberculosis, acute pulmonary infection.	
7. Liver diseases; Hepatitis, cirrhosis	
8. Miscellaneous; Pregnancy and perinatal, suicide, congenital anomalies.Burns, Diarrhoea/dysentery.	

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Mini Review - Evolutionary Aspects of Circadian Rhythm and Neurocardiovascular Dysfunction

R. B. Singh[1], Kuniaki Otsuka[2], Daniel Pella[*3], Jan Fedacko[3], Viola Michirova[3], Branislav Milovanovic[4], Krasimira Hristova[5], D. W. Wilson[6], and Hilton Chaves[7]

[1]Halberg Hospital and Research Institute, Moradabad, India
[2]Department of Internal Medicine, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan
[3]PJ Safaric University, Kosice, Slovakia
[4]Neurocardiological Laboratory, Department of Cardiology, University Clinical Center Bezanijska Kosa, Medical Faculty, University of Belgrade, Serbia
[5]National Heart Hospital, Sofia, Bulgaria
[6]School of Medicine, Pharmacy and Health, Durham, UK
[7]Federal University of Pernambuco, Recefe, Brazil

Introduction

Life on earth is governed by the 24 hour day and the continuous 24-hour cycle of light and darkness which appear to have definite influence on biological functions [1,2]. Time structure dependent synchronization of organisms with their environment is mediated by circadian clocks which is a cell autonomous mechanism identified within all cardiovascular-relevant cell types, including cardiomyocytes, endothelial cells as well as smooth muscle cells [1,2]. Physiological functions; blood pressures, hear rate, blood glucose, energy intake and expenditure also change, predictably throughout this 24 hour cycle, enabling us to anticipate predictable environment fluctuations over the day and to optimise the timing of various biological mechanisms to this 24 hour cycle [1,2]. Any disruption of this environmental cycle and/or the molecular mechanisms that interpret it add a third dimension to this equation resulting in to a dysfunction or development of a disease. The best example is blood pressure variation, but applies also to other mind-body mechanisms involved in cardiovascular homeostasis, such as the autonomic nervous system, electrophysiological myocardial function, myocardial metabolism, heart rate, endothelial function, haemostasis, as well as nutrient (salt, alcohol, antioxidants, vitamins, minerals, amino acids, lipid and carbohydrate) metabolism. Circadian misalignment due to environmental factors, has been implicated in the development of obesity, diabetes mellitus, and cardiovascular disease [1-3].

Halberg, the father of chronobiology, continue to educate what Barter said about circadian blood pressure variability [4,5]. Except to ascertain whether a patient is dead or alive, in all other cases, the clinician may gain from assessing chronobiologically the variability of heart rate and blood pressures,

[*] Correspondence: Daniel Pella, MD,PhD, FICC. Department of Internal Medicine, PJ Safaric University, Kosice, Slovakia, Daniel.pella@upjs.sk

electrophysiological functions and other vital signs that undergo a broad spectrum of rhythmic and other changes. Rodents are monitored to develop drugs, but not the humans for whom the drugs are intended. It took over half a century to begin to monitor blood sugar levels and to know the role of rapid glucose variations in the development of complications of diabetes mellitus. The role of primary risk factors; diet, physical activity, salt, stress and alcoholism, in patients with prehypertension or diabetes mellitus and its complications, particularly vascular diseases, needs further monitoring [1-4].

Monitoring blood pressure is equally timely, technically feasible for individual home- and website-based personalised care. Variations in blood pressures are related to time structure as well as to mind body connection [1-3].

Chronomedicine serves basic science and perhaps the management of societal illnesses; cardiovascular diseases (CVDs), diabetes mellitus, psychological disorders, degenerative brain diseases and cancer which are predisposed by primary risk factors and risk behaviours.

It is superfluous that hypertension experts around the world want to administer drugs to these subjects without knowing their exact blood pressures. It may be due to ignorance or convenience but indicate their business partnership with the company supplying the drugs. We have forgotten the old dictam that find out the cause of the disease and treat it. It is well known that the cause of hypertension lies in diet and lifestyle, so better is to change it for treatment of prehypertension.

Otsuka et al have reported the role of chronoecological health watch on arterial stiffness and neurocardiopulmonary function in elderly community at high altitude (3524m) compared with Japanese town indicating that living on high altitude may have adverse effects on health [6,7]. These adverse effects due to environmental factors may be modulated by antihypertensive foods and lifestyle rather than antihypertensive drugs because it is change in diet and lifestyle which are actual causes of CVDs [8,9].

In everyday life, the suprachiasmatic (SCN) and clock gene present in every living cell, contribute to the coordination of the circadian rhythms' phase and amplitude, (Figure 1) [1-4]. There is emergence of cardiovascular events, when our neuroendocrine time structures (chronomes) are not able to cope with the adverse effects of stimuli from within or from without, acting, e.g, *via* the sympathetic nervous system [2-4]. Circadian rhythms of various biomarkers of health have been observed after its discovery by Franz Halberg, which are under control of circadian clocks present in all body cells and under influence of master clock present in SCN [10,11].The evolution of circadian clocks have occurred to synchronize the physiology, metabolism and behavior to the 24-h geophysical cycles of the earth [1-3].

The words circadian and chronobiology were used for the first time by Franz Halberg in 1950 and officially introduced to a nomenclature committee in Stockholm for the first time in 1955 [10,11]. Chonobiology developed globally, after1969, when an article entitled Chronobiology was published in the Annual Review of Physiology and became a Current Content citation classics [10-12].

In the BIOCOS project, an extended consensus on need and means to detect vascular variability disorders (VVDs) and vascular variability syndromes (VVSs) have been proposed by Halberg and his followers (12). However, research, by Vanin et al. 2012 suggests that much of scientists' current understanding about *Drosophila* circadian behavioral rhythms may actually be wrong [13]. The experiment emphasizes the importance of studying organisms in natural environments and challenges 40 years of assumptions about circadian clocks studied in the laboratory [13].

The mystery of flies behaving in the wild suggest that the body clock in a wild, natural environment, shows features, that are impossible to observe in simplified laboratory conditions. It seems that the behavior of the fly in the wild is quite different from what is seen in controlled conditions in the laboratory.

The fly may be able to adapt its molecular mechanisms in the laboratory which is not its natural environment. This may be similar to men, when his blood pressure is measured in the physicians chamber rather than in his environment. It would be important to know the origin and evolution of circadian rhythm to understand its role in CVDs.

The Evolution of Circadian Rhythm and Cardiovascular Events

Circadian rhythm was known to ancient man from the time of *Homo erectus and Homo sapiens* who use to have intercourse in the early morning hours, before going for hunting to forests, causing increased release of testosterone in the morning to coordinate the sexual activity, which evolved as a circadian rhythm. This act was important to protect the progeny because life expectancy was not very high and early men died due to trauma in young age.

It has been also proposed that during human evolution, early man used to go for hunting near the river banks where animals come for drinking of water at sun rise [1,2]. The early man had marked excitement during running, climbing and may be horse riding while going for hunting, causing marked increase in mental and physical stress, with increased release of catecholamines and cortisol with excessive sweating [1,2]. These physiological and biochemical changes may have been responsible for the origin and the evolution of the circadian rhythm of increased sympathetic activity causing increased blood pressures, heart rate, breathing rate and endothelial dysfunction; the cardiovascular biomarkers which are known to predispose cardiovascular events as well as greater basal metabolic rate in the second quarter of 24 hour cycle. (Figure 1) [1,2].

Figure 1 indicates that refined carbohydrates, w-6 fatty acids may have adverse effects on circadian rhythms whereas w-3 fatty acids and antioxidants may have beneficial effects.

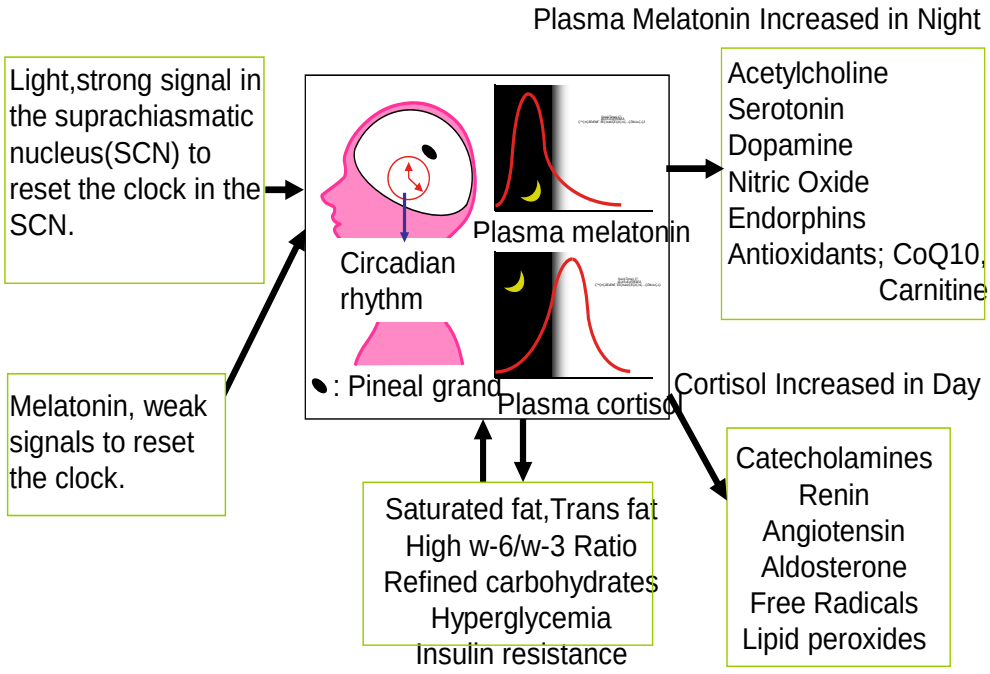
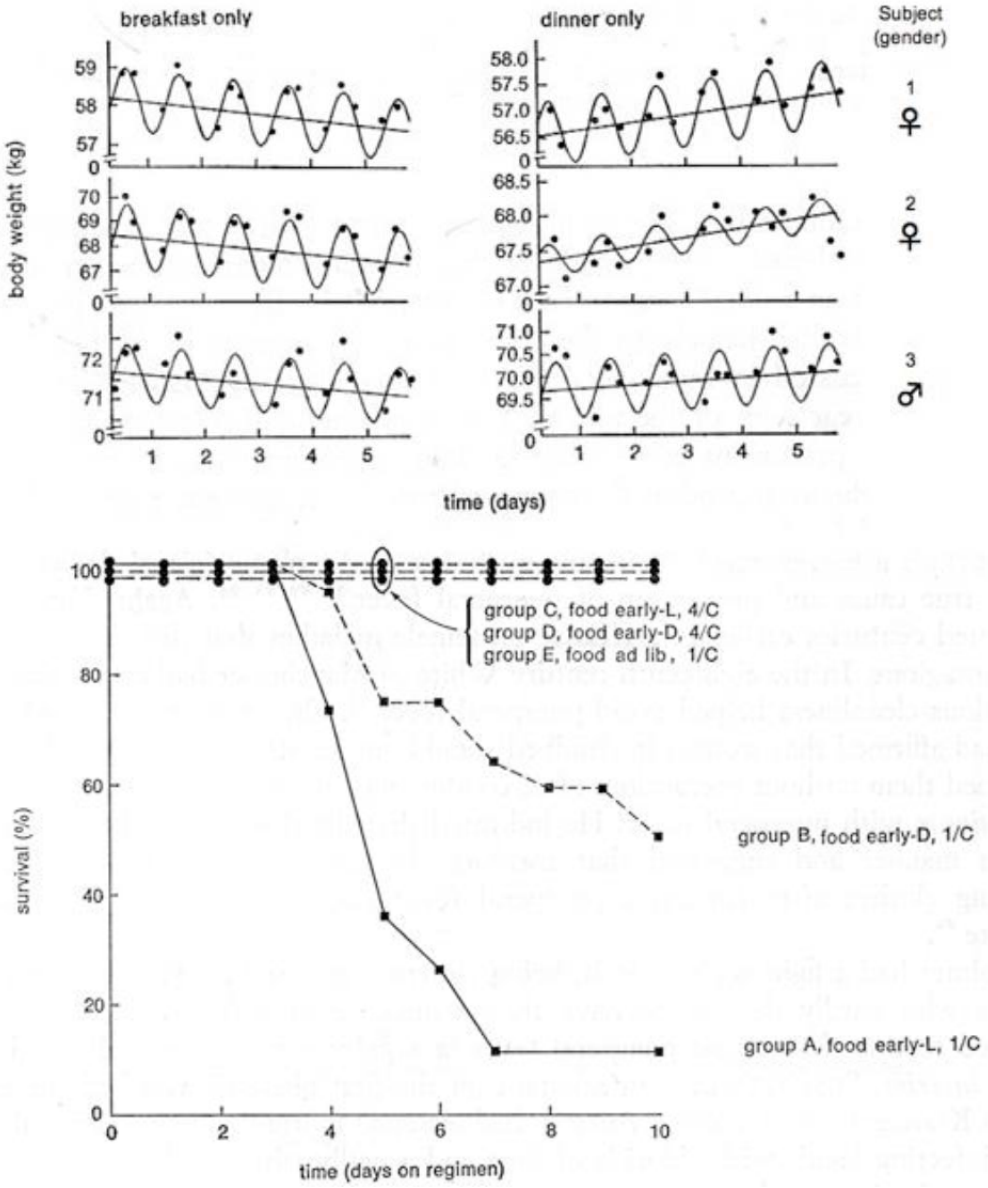


Figure 1. Control of daily phythms by molecular clock.

It is proposed that a circadian increase in basal metabolic rate in the morning as a circadian rhythm compared to evening, may be responsible for increased energy expenditure by the body during this period. Halberg utilized timing for meals in the morning and evening and demonstrated that eating same amount of energy in the morning causes decrease in body weight compared to evening causing increase in weight [14]. Recently, Cornelissen has reviewed "When you eat matters: 60 year,s of Franz Halberg, s nutrition chronomics ". [14]. Figure 2 shows the effect of feeding in the wrong time on weight gain in men and bottom on death or survival in mice [14].



Timing of calories determines outcomes (gain vs. loss in weight, top; deaths vs. survival, bottom).

Figure 2. Effect of feeding in the wrong time on weight gain in men and bottom on death or survival in mice.

These contributions have far-reaching implications, whether for feeding the under-nourished in populations stricken by starvation, or for managing one of today's great scourges in the more opulent countries, namely obesity. The use of a calorie consumed in the morning differs from that of one ingested in the evening, contributing, as he also showed, to different hormonal relations prevailing when a single daily meal is taken as breakfast-only or dinner-only. Important mechanisms underlying metabolism as they relate to health and longevity uncovered by Franz in the innumerable studies he designed and conducted on several continents remain beyond the frontier of current scientific investigations, attesting to his visionary leadership, his ability to look at the facts without any preconceived ideas, and his truly amazing humility and eagerness to serve.

The role of nutrition in chrono-cardiovascular dysfunction has also been reported by Singh and coworkers [15]. In one study among 209 adults, increased heart rate and MESOR blood pressures were observed in the second quarter of the day [16].

Fruit and vegetable intake were inversely associated with MESOR hypertension [17]. In another study measuring heart rate and blood pressure, half hourly for 7 days, larger circadian amplitude of heart rate associated with active prayer were observed in Hindu Indians in Asia [18]. The circadian rhythm of antioxidant vitamins and oxidative stress have also been observed to protect against the oxidative stress caused by catecholamine in the second quarter of the 24 h cycle [19].

Diet and lifestyle factors and geophysical cycles may trigger the circadian system; neuroendocrines and may activate the pineal gland, pituitary functions and adrenal secretions, resulting in adverse effects on circadian variations, heart rate variability(HRV) and blood pressure variability (BPV) sleep disorders, obesity and cardiovascular disturbances [1-3]. Circadian rhythms (24-hour) and also possibly cicaseptan rhythms of behavioral and physiological changes driven by oscillating biochemical mechanisms, are present in almost all organisms, including humans and even in some bacteria [1-3]. Many human health problems have a rhythmic component, including change in temperature, heart rate and blood pressures as well as other biomarkers as in cases of cancer and atherothrombosis [1,2]. Cornelissen et al proposed that Chronobiology predicts actual and proxy outcomes when dipping fails [20].

Exercise as a synchronizer of human circadian rhythms of coagulation and other biomarkers have been reported which may alter the natural course of circadian rhythms of cardiac dysfunctions [21]. One of our study shows that morning time exercise may be helpful in adaptation of our cardiovascular responses, causing less increase in MESOR hypertension and heart rate which may be protective against increased circadian rhythms of cardiovascular events [21]. A Mediterranean style diet rich in fruits, vegetables, nuts and w-3 fatty acids and flavonoids may be protective against the adverse effects of circadian rhythms of cardiac events [22]. In clinical studies circadian rhythms of cardiovascular events have been observed by several workers [1-4,15,23,24]. More recently, Wilson et al have published an interesting view point the origin of life and the evolution of circadian system [25]. Extended consensus on need and means to detect vascular variability disorders (VVDs), and vascular variability syndromes (VVSs) and the role of brain-body interactions are not yet popular among most experts although they are wisely described by Halberg [12]. Definition of ambulatory blood pressure targets for diagnosis and treatment of hypertension in relation to clinic blood pressures is based on 24-48 hours records which ignores the presence of circa-septan variability in blood pressures as well as VVS and VVDs indicating that 7 day record may be important preferably during working conditions to diagnose prehypertension or hypertension and premetabolic syndrome [12].

Concerning (internal) evolution within organisms as well as about an external one with their environment, a distinction has been made. Now Halberg make a third distinction, as we learn from Vernadsky's noosphere, which he regarded as a linear rather than cyclic entity, while we also think yet further scrutize the human mind's modification of the crust of the earth. Halberg also reported a transdisciplinary set of coperiodisms, a chrono-noosphere or rather, a chronousphere evolves. This is a feature of active rather than only passive bioevolution, a feature of cosmic evolution. There the problem of cyclic universes looms large.

In brief, circadian rhythm evolved with evolution of life but was discovered by Franz Halberg for the first time in relation to variability of various biological functions according to time structure resulting in to development of chronobiology, chronocardiology and chronotherapy.

Conflict of interest has not been declared by the authors.

Acknowledgments are due to International College of Nutrition and International College of Cardiology for the logistic support to write this article.

References

[1] Singh RB, Singh AK, Sharma JP, Singh RK, Kumar A, Rastogi SS, Singh G, Kartikey K, Singh S, Fedacko J, Pella D, De Meester F, Wilczynska, A; Wilson DW. Nutrition in Chronocardiology: We are Indebted Professor Franz Halberg. *The Open Nutr Jour,* 2012, *5,* (Suppl 1-M3) 45-65.

[2] Singh RB, Gupta A, Pella D, Circadian cardiology. *World Heart J* 2008; 1: 101-26.

[3] Otsuka K, Cornelissen G, HalbergF. Circadian rhythms and clinical chronobiology. *Biomed Pharmacother* 2001; 55: 7-18.

[4] Otsuka K,Shohgo M,Kozo M,Zi-yan Z,Weydahl A,Hansen TL. et al. Chronomes,aging and disease.In Furukawa H,Nishibuchi M,Kono Y,Kaida Y ediors; Ecological Destruction, Health and Development: *Advancing Asian Paradigms*,2004,p 367-94.

[5] Bartter F C. Periodicity and medicine. In: Scheving L E, Halberg F, Pauly J E (Eds) *Chronobiology*. Tokyo: Igaku Shoin Ltd, 1974; p 6-13.

[6] Otsuka K, Cornelissen G, Norboo T, et al. Chronomics and glocal assessment of human life. *Prog Thera Phys* 2008;Supple 173;134-152.

[7] Otsuka K, Norboo T, Otsuka Y et al. Chronoecological health watch of arterial stiffness and neurocardiopulmonar function in elderly community at high altitude (3524m) compared with Japanese town. *Biomed Pharmacother* 2005;59:Supple 58-67.

[8] Singh RB, Rastogi SS, Sircar AR, Mani UV, Singh NK, Niaz MA. Effects of lifestyle changes on atherosclerotic risk factors in the Indian Diet Heart Study. *Amer J Cardiol* 1993; 71: 1283-88.

[9] Singh RB, Rastogi SS, Singh R, Ghosh S, Niaz MA. Effects of guava intake on serum total and high density lipoprotein cholesterol levels and systemic blood pressures. *Amer J Cardiol* 1992; 70: 1287-91.

[10] Halberg F, Stephens AN. Susceptibility to quabain and physiologic circadian periodicity. *Proc MinnacadSci* 1959; 27: 139-43.

[11] Halberg F, Visscher MB. Regular diurnal physiological variation in eosinophil levels in five stocks of mice. *Proc Soc Exp Biol* (NY) 1950; 75: 846-7.

[12] Halberg F, Cornelissen G, Otsuka K, Siegelova J, Fiser B, Dusek J, Homolka P, Sanchez de la Pena S, Singh RB, BIOCOS project. Extended consensus on need and means to detect vascular variability disorders (VVDs) and vascular variability syndromes (VVSs). *World Heart J* 2010; 2 (4): 279-305.

[13] Vanin S, Bhutani S, Montelli S, Menegazzi P, Green EW, Pegararo M, Sandrelli F, Costa R, Kyriacou CP. Unexpected features of Drosophila circadian behavioural rhythms under natural conditions ," *Nature,* 2012; doi:10.1038/nature10991, 2012.

[14] Cornelissen G. When you eat matters: 60 year,s of Franz Halberg, s nutrition chronomics. *The Open Nutr J* 2012;4:16-44.

[15] Singh RB, Cornelissen G, Weydahl A, Schwartzkopft O, Katinas G, Otsuka K et al, Circadian heart rate and blood pressure variability considered for research and patient care. *Int J Cardiol* 2003,87:9-28.

[16] Singh RB, Cornelissen G, Siegelova J, Halberg F. About half weekly pattern of blood pressure and heart rate in men and women of India. *Scripta Medica* (BRONO) 2002; 75: 125-128.

[17] Singh RB, Cornelissen G, Otsuka K, Juneja L, Halberg F. Coronary risk factors, ambulatory blood pressures and heart rate in Asian Indians. *The Open Nutra J* 2012;5:79-80.

[18] Singh RB, Cornelissen G, Kumar A, Bathina S, Halberg F. Larger Circadian Amplitude of Heart Rate Associated with Active Prayer in Hindu Indians in Asia. *World Heart J* 2009;1: pp. 219-222.

[19] Singh RB, Niaz MA, Cornelissen G, Otsuka K, Siegelova J,Fiser B, Halberg F. Circadian rhythmicity of circulating vitamin concentrations. *Scripta Medica* (BRONO),2001,74:93-96.

[20] Cornelissen G, Halberg F, Otsuka K, Singh RB, Chen CH. Chronobiology predicts actual and proxy outcomes when dipping fails. *Hypertension* 2007; 49:237-39.

[21] Singh RB, Halberg F, Cornelissen G, Siegelova J, Hristova J, Toda E, Takahashi T, Jan Fedacko J, Otsuka K. Personalized circadian timing of exercise. *World Heart J* 2013,5:79-90.

[22] Gal D,Pella D, Singh RB. The effect of an alpha-linolenic acid rich diet on the circadian rhythm of cardiac events. *World Heart J* 2008;1:49-56.

[23] Singh RB, Pella D,Rastogi S,Sharma JP,K Kartikey, Goyal VK et al. Increased concentrations of lipoprotein(a),circadian rhythms,and metabolic reactants evoked by acute myocardial infarction in relation to large breakfast. *Biomed Pharmacother* 2004, 58(Supple):116-122.

[24] Singh RB, Pella D,Neki NS, Chandel JP, Gupta P, Rastogi S et al. Mechanism of acute myocardial infarction(MAMI) study. *Biomed Pharmacother,* 2004,58(Supple):111-115.

[25] Wilson DW, De Meester F, Singh RB, Gerasimova E. The origin of life and the evolution of circadian system. *World Heart J* 2013;5:121-130.

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Gender-Specific Issues in Coronary Artery Disease*

Adarsh Kumar[#1],
Harharpreet Kaur[2],
R. B. Singh[3],
Germaine Cornelissen-Guilaume[4],
and Ankita Attri[5]

[1]Professor and Head of Cardiology Department, Govt. Medical College/GND Hospital, Amritsar, Punjab, India
[2]Professor, Medicine Department, Govt. Medical College, Patiala, India
[3]Dr R. B. Singh, Honorary Professor, Preventive Cardiology, Medical Hospital and Research Centre, Civil Lines, Moradabad, India
[4]Professor, Halberg Chronobiology Center, University of Minnesota, USA
[5]Resident Doctor, GMC Amritsar

Abstract

The popular perception that Coronary Artery Disease (CAD) is a man's disease is more of a myth than reality. It is the leading cause of death in adult women. Women with coronary artery disease have a worse outcome as compared with their male counterparts especially the younger ones and should be treated aggressively. CAD in women has certain unique features. Symptoms appear 10 years later in women than in men. The diagnosis is often missed due to the atypical clinical presentations. It has been observed that women commonly have normal coronaries on angiography but that does not mean that it is a benign condition. Inflammation, microvascular disease, endothelial dysfunction, coronary spasm and plaque erosion probably play an important role in the pathogenesis of ischemic heart disease in women. In addition their vessels are smaller with impaired vasodilator responses and lesser collaterals explaining the unfavorable outcome. Non ST elevation syndromes are more common than STEMI in women. Awareness of these peculiarities can help in early diagnosis and timely treatment. There is need to develop newer gender specific diagnostic strategies to detect ischemia in women and to plan appropriate treatment modalities.

Keywords: Coronary artery Disease, Women

1. Introduction

It is a misconception that Coronary Artery Disease (CAD) is less common and less severe in women. The vague discomfort experienced in the chest by women is often mistaken for indigestion .Not only the women but their health care providers also underestimate this risk with consequent absent or suboptimal recommendations for coronary disease prevention and treatment. During the reproductive age women do have a lower incidence of CAD, but after menopause the incidence is the same or higher. Between 45 to 64 years of age, one in nine women develop some form of heart disease. After age of 65, the ratio climbs to one in three.

* This article is dedicated to the memory of Professor Franz Halberg, the father of chronobiology and our revered teacher.

Address for correspondence: Adarsh Kumar, MD, DM, FACC, 17 F Beauty Avenue, Phase 2 Amritsar, Punjab, INDIA. Telephone: 01832500707 Mobile: 0919501523399. Email: adarshkumar_27@yahoo.com

It is a leading cause of death for women throughout the world. Since 1984, more women than men have died of heart disease. But in recent years, women, particularly the younger ones have experienced improvements in hospital mortality.[1] In one recent study whereas after ST elevation myocardial infarction (MI) the short term mortality was the same in men and women, after Non ST elevation MI and unstable angina it was lower in women after correction for age and risk factors. [2] However this is not a signal that all is well. Women are still at an immense risk from CAD.

Compared with men, they have worse outcomes after myocardial infarction. [3] They have more persistent symptoms, more episodes of angina, more frequent hospitalizations and higher health care costs. [4] At presentation to hospital women with MI are more likely to have a higher Killip class, tachycardia, atrioventricular block, and pulmonary rales. Complications including shock, heart failure, cardiac rupture and stroke are more common in women; although these gender differences lessen with correction for comorbidity and older age. [5] Women especially the younger ones also have higher rates of sudden cardiac death prior to hospital arrival compared with men.[6] Paradoxically despite higher rates of myocardial ischemia women have less extensive and less severe anatomical obstructive coronary artery disease. [7] They are more prone to have diastolic heart failure. In fact Coronary artery disease in women not only differs in the age of manifestation but also has certain unique features with regard to pathophysiology , clinical presentations, risk factors and investigation modalities Table 1 & 2.

The reasons for these discrepancies are poorly understood and many theories have been proposed.

Table 1. Major gender differences in coronary artery disease with regard to pathophysiology and investigation modalities

	Females	**Males**
Pathophysiology	Diffuse plaque more common	Focal Obstruction more common
	Normal coronary angiograms in 20-30% patients.	Normal coronary angiograms in 6-10%
	Microvascular disease more common	Microvascular disease less common
	Coronary vasospasm more common	Coronary vasospasm less common
	Smaller vessels, impaired vasodilator responses, lesser collaterals,endothelial dysfunction more common	Less common
	Plaque erosion more common	Plaque rupture more common
	Lesser atheroma volume	More atheroma volume
Investigations	Exercise stress testing the specificity and positive predictive value are significantly lower in women	Specificity and positive predictive value are comparatively more.
	Cardiovascular magnetic resonance more useful to detect above abnormalities	Comparatively less useful
	Women are more likely to have elevated C-reactive protein (hs CRP) and Brain Natriuretic Peptide (BNP).	Men are more likely to have elevated creatine kinase MB and troponins

Table 2. Major gender differences in coronary artery disease with regard to risk factors, social factors and clinical presentation

	Females	**Males**
Risk Factors	Menopause and birth control pills along with smoking risk factors in women	No
	Diabetic women have significantly higher IHD mortality and cardiovascular complications as compared with diabetic men.	Comparatively less significant
	Hypertriglyceridemia is a more potent independent risk factor for women as compared to men	Comparatively less significant
	Truncal obesity and increased body mass index (BMI) have recently been proposed as potential independent risk factors	Comparatively less significant
	Systolic blood pressure rises more steeply in ageing women compared with men	Comparatively less significant

	Females	**Males**
Social factors	Generally receive suboptimal and less-aggressive therapy following cardiac ischemic events especially in India	Receive adequate therapy
Clinical presentations	Women present more frequently with non–ST-elevation myocardial infarction (NSTEMI)	Men often have ACS with ST elevation (STEMI) on presentation with a much less marked sex difference among older patients
	Stable angina (47% in women compared to 26% in men)	
	Anginal equivalent shortness of breath more common	Less common
	Nausea, indigestion, gastrointestinal symptoms and dizziness more common	Less common
	Sense of constriction in the chest , throat ,jaw or neck and back pain more common presentation	Less common
	Diffuse discomfort more common	classic crushing chest pain more common

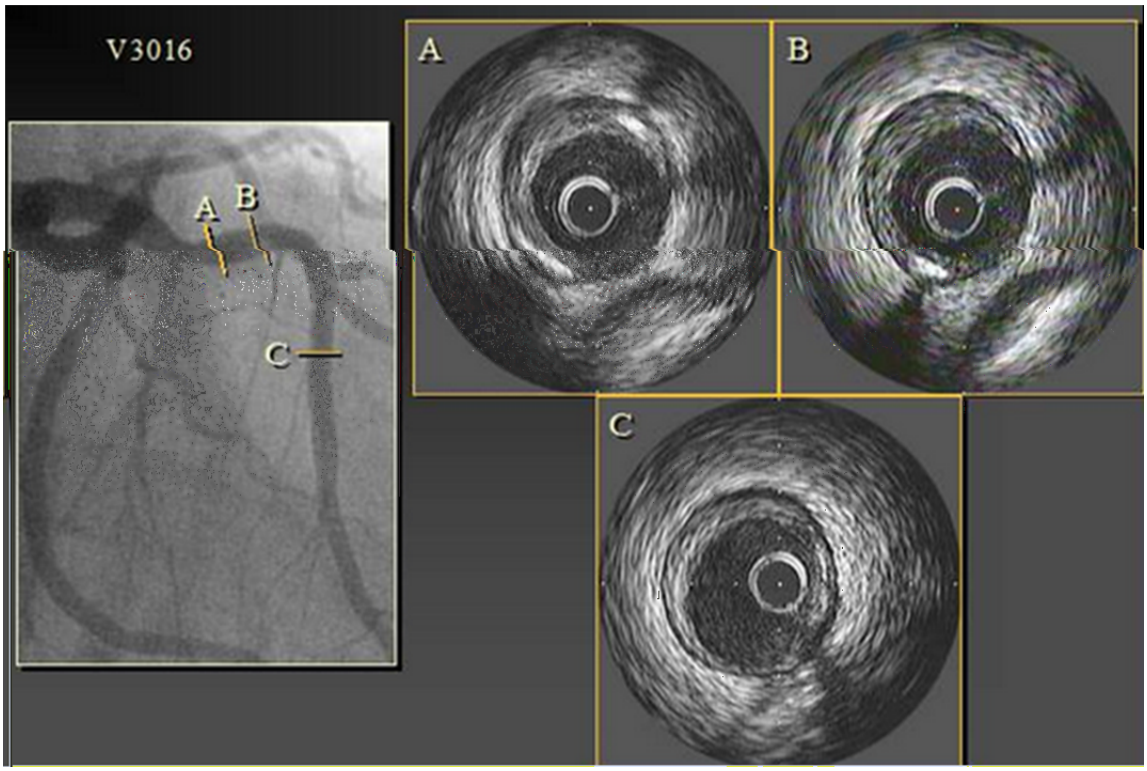


Figure 1. Progression of plaque disease without luminal narrowing.

2. Pathophysiological Mechanisms of Ischemia in Women

2.1. Normal Angiograms

In Acute Coronary Syndrome (ACS) /ST segment elevation MI (STEMI), 20-30% women as compared to 6-10% men have "Normal" coronary angiograms, defined as no visible obstructive CAD or luminal irregularities and <50% stenosis. [8] In one study it was observed that 20% to 30% of women with troponin-positive acute coronary syndrome had no obstructive coronary artery lesions.[9] The 1970s Coronary Artery Surgery Study included women, who made up about a quarter of the 25,000 participants and for the first time observed that a woman with a positive stress test was 4.5 times more likely than a

man with a positive stress test to have an angiogram without any obstructive lesion. It was initially concluded that the stress test is less reliable in women. However it was observed that even women whose coronary arteries were clear continued to die from ischemic heart disease (IHD). In 1996, the National Institutes of Health (NIH) decided that these gender disparities couldn't be ignored any longer and decided to undertake the Women's Ischemia Syndrome Evaluation (WISE) study which was designed to optimize symptom evaluation and diagnostic testing in IHD and to explore mechanisms for myocardial ischemia in the absence of epicardial coronary artery stenoses. The WISE study results have advanced our understanding of the nature of myocardial ischemia in women and also explained the normal coronary angiograms. As expected, many symptomatic women with positive stress tests had negative angiograms. But many of these women had abnormal results on vascular function tests. It was concluded that functional rather than structural abnormalities of the coronary circulation were possibly implicated in causing ischemia in women. [10]

2.2. Microvascular Dysfunction, Vascular Inflammation and Endothelial Dysfunction

Other explanations put forward to explain the clean coronaries with excess mortality and morbidity include increased prevalence of microvascular disease in women. Microvascular Coronary Dysfunction (MCD) is a condition affecting the pre-capillary coronary arterioles, which branch from the epicardial coronary arteries, but occur before the capillaries. It is defined as a limited coronary flow reserve and/or coronary endothelial dysfunction and is thought to be the predominant etiologic mechanism of ischemia in women with the triad of persistent chest pain, non-obstructive coronary artery disease and ischemia evidenced by stress testing.

Microvascular dysfunction may include the following alone or in combination: (1) Altered resting vascular smooth muscle tone secondary to either smooth muscle cell dysfunction or abnormalities of the vascular endothelial cells, which are unable to produce enough nitric oxide leading to suboptimal dilatation . (2) Non-endothelial auto-regulation of the micro vasculature with altered responses to constrictor or dilator stimuli; (3) reduced number of arterioles and capillaries (eg, rarefaction); and (4) structural alterations that contribute to decreased lumen size, increased wall-to-lumen ratio, increased stiffness and remodeling. The presence of endothelial dysfunction in women and differences in vasodilatory reserve has been refuted by some later studies. [11]

2.3. Coronary Reactivity

Women suffer disproportionately from a variety of generalized vascular disorders, including migraine headaches, Raynaud's phenomenon and autoimmune arteritis etc. The WISE study also considered the issue of abnormal smooth muscle contraction in the epicardial coronary arteries. Recent evidence is suggestive that coronary vasospasm may be the dominant etiology of acute myocardial ischemia in women. This may explain the poor sensitivity and specificity of the routine myocardial perfusion tests because this spasm may be transient.

The role of coronary reactivity in Prinzemetal's angina is well known and is characterized by abnormal proximal epicardial coronary artery vasospasm modulated by smooth muscle dysfunction. It is now clear that constriction of intra-myocardial microvascular arteries mediated by endothelial and autonomic nervous system adrenergic pathways are also involved in producing ischemia. [12,13].

Coronary spasm especially in vessels with a non-obstructive atheroma can cause myocardial infarction and sudden death [14,15]. It is notable that pathologic evidence of MI may exist in the absence of obstructive CAD in women. [16] and may be due to the causes explained above. In most studies, more than 90 percent of patients with stress cardiomyopathy are women, a condition associated with high levels of adrenalin, irregular or fast heart beating and heart failure. Fortunately, these are most often reversible. Tragically, the condition may result in sudden death in the most extreme cases—"She was scared to death."Furthermore the acute stress-induced cardiomyopathy (Tako-Tsubo disease) is more than nine times prevalent in older women than in men and may occur more frequently than it is currently

diagnosed. [17] It is thought to be due to coronary vasospasm and microvascular dysfunction.

Thus coronary spasm may play an important role in producing ischemia in women. Indeed, a recent study identified provoked coronary artery spasm in 50% to 70% of acute coronary syndrome patients without obstructive lesions. [18].

2.4. Smaller Vessels with Lesser Collateral Circulation

Besides the functional abnormalities, on an average, women have vessels 10% smaller than men's with lesser collateral circulation leading to increased incidence of ischemia during increased myocardial work. These vessels frequently show impaired vasodilator responses. Therefore an area of ischemic injury can extend because the usual vasorelaxation required for collateral function is abnormal. This theory could help explain why women tolerate Acute Coronary Syndromes poorly compared with men.

The assessment of Non obstructive Coronary Artery Disease (NOCAD is not well established. The gold standard test to diagnose Microvascular Coronary Dysfunction (MCD) is an invasive coronary reactivity testing. [19] Coronary vasospastic angina can be diagnosed by the acetylcholine-provocation test during coronary angiography, and microvascular coronary spasm can be diagnosed by measurements of coronary blood flow and myocardial lactate production during acetylcholine-provocation test. Microcirculatory insufficiency and diffuse arteriosclerosis can be evaluated by the measurement of coronary blood flow during adenosine-provocation and by stress myocardial scintigraphy. [10] Imaging techniques like CMR and SPECT evaluate left ventricular myocardial blood flow, which is downstream of both obstructive CAD and microvascular dysfunction. The subendocardium is most susceptible to perfusion impairment and ischemia occurs in the subendocardium before advancing to the subepicardial layer. Subendocardial perfusion imaging may therefore be an important technique to enhance the sensitivity for detection of myocardial ischaemia especially in women.

Digital Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT) has been investigated in predicting ischemic heart disease (IHD), including obstructive coronary artery disease (CAD) and nonobstructive coronary artery disease in women and found that RH-PAT noninvasively predicted the presence of IHD, especially NOCAD, before coronary angiography. RH-PAT is a potentially useful clinical test and can effectively help to identify high-risk women with chest pain. [20].

2.5. Female-Pattern of Atherosclerotic CAD

Coronary artery disease in women is very different from coronary disease in men. This illness in men produces focal obstructions of the artery that cause chest pain with exercise that is relieved by rest. Female pattern of CAD is a poorly recognized form of atherosclerotic CAD that is seen mainly in women. Structurally, women's coronary vessels more commonly have a more diffuse atherosclerosis with involvement of the entire circumference of the artery, and not just localized plaques. This cholesterol plaque is distributed evenly throughout the arterial system producing arteries that are small and with less focal obstruction. Still, these plaques can rupture and produce clot. Not only is the plaque deposition in women diffuse, but it remodels the entire artery outward and the lining of the artery becomes thickened throughout, making the plaques flush with the wall of the vessel . The atherosclerotic lesion protrudes outward rather than impinging on the lumen.[21] They may therefore be very large before producing any obstruction. This diffuse, vulnerable plaque in women explains why women with repeated chest pain in the WISE study still had a high risk of heart attack and other cardiovascular events, even with a normal heart catheterization. There is evidence that coronary vessels of women with IHD are more diseased compared to men [22].

2.6. Plaque Lesions with Erosion and Distal Embolisation

In women, the plaque is more likely to be deposited uniformly around the inside of the vessel. The stiff interior walls tend not to rupture under stress but rather, to erode. It may be produced as a result of

vasospasm. In erosion pathology the endothelial cells on the vessel wall are eroded, leaving a raw surface that causes a thrombus to form in the lumen of the artery. This may be just as, if not more, important as the plaque rupture. Plaques with endothelial loss and surface erosion are associated with clusters of smooth muscle cells and proteoglycans at the luminal surface and may be similarly vulnerable to acute thrombosis as are lipid-rich plaques. [23]. Plaque erosions are the predominant abnormality in premenopausal women who die suddenly especially those who smoke whereas rupture of plaques is more common in older women and in men. [21]. Plaques with these superficial erosions do not, by themselves, cause critical obstruction but can still result in sudden death. [23] In such cases, the coronary obstructions are precipitated largely by the thrombi that develop on the dysfunctional intima.

In one study in plaque ruptures on pathological examination nearly one-half of the thrombi were classed as being in early stage of maturation, with the remaining (50%) categorized as at late stage. In contrast coronary thrombi in fatal erosions are often in later stages of maturation as compared with ruptures. [24] However this greater thrombus organization in erosion occurs in the deeper regions of the thrombus where it mixes with plaque but there is persistence of platelet aggregates near the luminal surface, where this dynamic process might constitute a persistent nidus for distal embolization. Microemboli and arrhythmia due to ischemia may be the final triggers of sudden death in plaque erosions.

Intravascular ultrasound studies (IVUS) have demonstrated that in minimal angiographic coronary artery lesions, there is often extensive atherosclerosis commonly obscured by remodelling of the arteries. The lesions do not impinge on the lumen. Plaque rupture was the most common finding but erosions were also seen. Evidence for plaque disruption by IVUS was present in almost 40% of women with ACS and nonobstructive CAD or "normal angiograms." Since angiography was normal in these cases it was hypothesized that plaque disruption as observed in these studies on intravascular ultrasound was accompanied by either a distal embolization of atherothrombotic debris or platelet aggregates or by transient thrombosis with endogenous thrombolysis, leading to widely patent arteries by the time of an angiography done a median of two days after symptom onset. [25]. Half of the women (11 of 22) without IVUS evidence of plaque disruption showed an ischemic pattern on cardiovascular magnetic resonance (CMR). Spasm could have accounted for an ischemic pattern on CMR, particularly in the absence of plaque rupture. [26].

In women with angiographic coronary artery disease a lower atheroma volume than in men, including both intraluminal plaque and atheroma within the media, has been reported. [27]

2.7. Combined Effect of all Factors

It is apparent that more than one of the above factors can combine to produce ischemia. Overall there is less plaque burden in women with coronary artery disease which is prone to erosion, inflammation and thrombosis. The intimal plaque not only changes the vessel geometry but also interferes with functions of the endothelium, including vasodilation.

Microvascular dysfunction would have the potential to amplify the consequences of an upstream spastic or thrombotic event. The concept of coronary artery spasms with minimal atherosclerosis has been introduced. Disruption of the plaque when coupled with vasospasm and microvascular disease could all play a part in producing ischemia in women. [28] Thus ischemic heart disease may be a more appropriate term rather than coronary artery disease. However more research is needed to support these theories.

2.8. Role of Circadian Variations in Ischemic Activity in Women

Biological timekeepers exist which are genetically controlled, and are important factors in the biology of organisms. Blood pressure fluctuates rhythmically. [29,30,31]. There is circadian variation in coronary events and sudden cardiac death. Episodes of prinzmetal angina and angina associated with ST depression show circadian variation with peak occurance in the morning and 1 to 3 hours after awakening. The circadian variations in transient ischemic activity, mean heart rate and ischemic

threshold have been compared in women and men with coronary artery disease and it was found that there was a similar and significant circadian variation in ischemic activity in both men and women. There is lower nocturnal ischemic threshold due to lower blood pressure consistent with circadian variation of coronary vascular tone [32].

3. Types of Coronary Syndromes in Women

The duration and severity of blood flow impairment determines the severity of the acute coronary syndrome which could range from unstable angina to myocardial infarction (MI).[28] Women present more frequently with non–ST-elevation myocardial infarction (NSTEMI), whereas men often have ACS with ST elevation (STEMI) on presentation with a much less marked sex difference among older patients. [33,34]. This finding may reflect different pathophysiologic processes, with ST elevation being secondary to occlusive thrombus and unstable angina reflecting subtotal occlusion. According to the Framingam Heart study the initial manifestation of CAD was more commonly stable angina (47% in women compared to 26% in men) which carries a good prognosis due to women's lesser angiographic disease burden .It is to be noted that if women do have MI, consequences are quite serious especially in the younger women.

4. CAD in Younger Women

The pathophysiology of coronary heart disease in premenopausal or middle-aged women may differ from the disease of older women and of men. Younger women under 65 years of age, who have a myocardial infarction (MI), have poorer outcomes as compared to men. Women less than 50 years of age are more than twice as likely to die during hospitalization than men in that age group.[35] Strikingly increased incidence of sudden cardiac death before reaching the hospital also has been reported in younger women aged 35 to 44 years. These women who die suddenly of coronary thrombosis are often cigarette smokers. They may have a hypercoagulable state, coronary spasms or microvascular disease with plaque erosion with lesser coronary narrowing & plaque calcium. Women beyond 65 who die suddenly of coronary thrombosis have high cholesterol, have plaque rupture with severe coronary narrowing and far more plaque calcium as in case of men and there is no differences in the mortality rate as compared to men. [5] It is possible that sex differences in vessel size, vasodilator responses and inadequate collateralization especially in the younger women who are totally unprepared are responsible for unfavorable outcomes after STEMI. In a recent study single vessel coronary disease was found to be the most common coronary angiographic feature of young women with AMI [36].

5. Risk Factors in Women

Women who present with IHD are more likely to have same risk factors like hypertension, diabetes or dyslipidemia as compared to their male counterparts. Though the risk factors are virtually the same in both sexes their impact is much more for women than for men.

Elderly hypertensive women are particularly at risk for CAD. Systolic blood pressure rises more steeply in ageing women compared with men. But even moderate or borderline hypertension (≥140/90mmHg) causes more endothelial dysfunction and cardiovascular complications in females than in men. Smoking especially in younger women especially in combination with oral contraceptives poses a 13-fold increase in CAD mortality. [37].

Diabetic women have significantly higher IHD mortality and cardiovascular complications as compared with diabetic men. [38]. Further, in women it has been shown that type 2 diabetes is a potent, independent risk factor for heart failure which cannot be fully explained by coexisting cardiovascular risk factors or previous myocardial infarctions.

Women beyond 50 have higher (average) blood cholesterol levels. Hypertriglyceridemia is a more potent independent risk factor for women as compared to men. The metabolic syndrome is an important risk factor in women. When focusing on gender aspects of the metabolic syndrome (MetS), the relative risk of insulin resistance, hypertension, and

elevated C-reactive protein levels is higher in women than in men [39].

Truncal obesity and increased body mass index (BMI) have recently been proposed as potential independent risk factors, particularly in young women with CAD. Obesity is prevalent in one-third of women. Postmenopausal status is associated with higher prevalence of obesity, as 44% of postmenopausal women are overweight, among whom 23% are obese. Moreover, estrogen withdrawal during menopause has a detrimental effect on metabolism and brings changes in body fat distribution from a gynacoid to an android pattern, causes reduced glucose tolerance, abnormal plasma lipids, increased blood pressure, increased sympathetic tone, endothelial dysfunction and vascular inflammation. Thus clustering of risk factors is common in women after menopause, especially the combination of obesity, dyslipidemia and hypertension and is potentially related to hormonally-mediated metabolic disturbances. It is to be emphasized that after menopause women need to pay extra attention to their health and fitness to prevent coronary artery disease.

Disruption of ovulatory cycling, indicated by estrogen deficiency and hypothalamic dysfunction or irregular menstrual cycling in premenopausal women is associated with an increased risk of coronary atherosclerosis and adverse CVD events [22].

Most of the younger women who have angina are obese, hypertensive, diabetic or dyslipidemic. A strong family history of cardiac disease may lead to manifestation of CAD at a younger age. Lower hemoglobin and uric acid are risk factors of young women with AMI. Complications during pregnancy such as preeclampsia and gestational diabetes are also important risk factors in a woman's personal history.

Patients with hypercoagulable states and certain connective tissue or collagen vascular disorders comprise a population also at high risk for early ischemic events. Women with rheumatoid arthritis usually have hyperlipidemia and are receiving steroids which predispose them to CAD. It is also important to screen these women for hypothyroidism who are also often and prone to atherosclerosis. Other younger women presenting with angina give history of hysterectomy. Psychological stress may lead to CAD in younger women. Mental stress, anxiety and panic disorders can induce endothelial dysfunction. Mental stress has also been linked with increased adrenergic outflow, vasoconstriction and impaired myocardial blood flow and accelerated coronary atherosclerosis progression especially in relatively young women.

6. Symptoms of CAD in Women

Almost half of the women with MIs present with shortness of breath, nausea, indigestion, burning sensation or dizziness. They are more likely to complain of a sense of constriction in the chest, throat, jaw or neck and back pain compared with men. They are known to undergo dental extractions or investigations for dysphagia for these vague symptoms due to lack of awareness. Breathlessness is a common angina equivalent in women which is mistaken for asthma or heart failure. Lastly a complaint of palpitation or a sense of uneasiness should not be dismissed off lightly in female patients.

Microvessel disease could also help explain why so few women have the classic crushing chest pain that signals coronary artery disease. Instead, they feel diffuse discomfort, exhaustion, or shortness of breath under stress or even during daily routines. Symptoms are nonspecific and less dramatic and may be termed as microvascular angina. [22].

Women exhibit a greater symptom burden and more functional disability. To confuse the issue is the fact that they also have non cardiac pain especially oesophagitis etc. quite frequently. Any women with vague symptoms like the ones described above and chest discomfort especially brought on by exercise and in the presence of risk factors mentioned above should be investigated for coronary artery disease.

7. Diagnosis of Myocardial Ischemia in Women

As coronary angiography may be negative in women with myocardial ischemia noninvasive diagnostic testing offers the potential to identify women at increased CAD risk as the basis for instituting preventive and therapeutic interventions. Steps should be taken to identify women at the

earliest stage of presentation so that appropriate therapeutic strategies can be implemented.

7.1. Exercise Stress Testing for Myocardial Ischemia in Women

Currently, exercise stress testing is one of the most commonly used method of diagnosing CAD in women. It is the initial noninvasive method for testing women with a normal resting 12-lead electrocardiogram (ECG) who are at intermediate risk for CAD based on risk factors and symptoms and are capable of maximal exercise stress. [40]. Whereas the sensitivity and negative predictive value of exercise stress testing are similar in men and women, the specificity and positive predictive value are significantly lower in women. The positive-predictive value of the exercise stress test is lower for women due to earlier fatigue and an impaired ability to reach target heart rate. In addition in premenopausal women, endogenous estrogen may have a digoxin-like effect during the stress test leading to ST segment changes, resulting in false positive results. Although women are more likely to have a "false-positive" exercise ECG, a negative exercise stress test is useful in effectively ruling out a diagnosis of CAD. If their ischemia is caused by coronary vasospasm the test may be negative unless it occurs during exercise. One of our patients had a sudden 3mm ST depression of horizontal nature during exercise testing which recovered in less than a minute .Later the angiography was normal and we were faced with a lawsuit. It is possible that she had a transient vasospasm.

Marked ST-segment changes (i.e., ≥2-mm) horizontal or downsloping ST depression at low workloads and persisting into recovery are all sensitive markers for the presence of obstructive CAD in women and men .Transient and upsloping ST changes with good exercise tolerance can be ignored if H/O angina is not typical and no risk factor is present.

However in addition to other important prognostic markers, exercise capacity (fitness level) can be estimated using an exercise stress test, and an exercise capacity of <5 METs or the inability to achieve ≥ 85% of age-predicted heart rate are important parameters. Fitness level has been shown to be a predictor of MI, IHD death, and all-cause mortality in women. Obstructive disease is more prevalent in women with a high Duke treadmill score (DTS) (defined as exercise time − (5 × ST deviation) − (4 × chest pain [1=nonlimiting, 2=limiting]) and these women may benefit from referral to coronary angiography. Women with an intermediate Duke treadmill score should, in general, be referred for additional risk stratification with a cardiac imaging study [41]. In women, exercise capacity, percentage of age-predicted exercise capacity, chronotropic response, HRR, blood pressure response, and the DTS can all be used to enhance the diagnostic and prognostic value of exercise ECG.

7.2. Stress Echocardiography

Stress echocardiography, with exercise or dobutamine pharmacologic stress, can be used to identify stress-induced ischemia. By providing information on the presence and location of wall-motion abnormalities related to a decrease in regional myocardial blood flow, the extent and location of ischemia can be defined. In symptomatic women who are incapable of exercise, dobutamine stress echocardiography reliably detects multivessel disease, with reported sensitivities from 75% to 93% and specificities of 79% to 92% .It may be limited by the variability in acoustic windows and the ability to capture images at the point of maximal stress. It has somewhat lower sensitivity for detection of intermediate stenosis or single-vessel CAD, but its high negative predictive value makes this a particularly useful test to rule out IHD in younger women [40]. However it is possible that this may not include patients who had experienced coronary vasospasm.

7.3. SPECT Myocardial Perfusion Imaging (MPI)

Stress-induced changes in myocardial perfusion have been extensively evaluated in women, largely employing SPECT imaging with more recent use of positron emission tomography (PET) and cardiovascular magnetic resonance (CMR) techniques. SPECT with ECG gating, a nuclear-based technique, provides quantitative information on

myocardial perfusion, regional and global left ventricular function. SPECT MPI with pharmacologic stress is useful because at-risk women are generally older and often have decreased exercise capacity when they present with symptoms of IHD. There is a large body of evidence relating to SPECT stress imaging showing that it effectively risk stratifies women with suspected IHD. In women with a normal myocardial perfusion study using SPECT imaging, the annual IHD event rate is very low, in contrast to a much higher event rate in those with abnormal myocardial perfusion.

There are some limitations to SPECT imaging in women, including (1) reduced sensitivity as a result of severe multivessel disease, or as a result of diffuse endothelial or microvascular disease; (2) limited resolution, where smaller abnormalities are undetected due to a smaller heart; (3) breast attenuation; and (4) radiation exposure [42]

7.4. Positron Emission Tomography (PET) Scanning

Positron Emission Tomography allows the measurement of average Cardiac Blood Flow per gram of myocardial tissue and therefore can be applied to identify Coronary Microvascular Disease. MPI with PET provides higher spatial resolution than SPECT, and is a powerful noninvasive modality for the diagnosis and risk assessment of CAD. PET eliminates attenuation artifacts that are problematic in SPECT imaging and appears to be more accurate in women. [43] However it has traditionally been perceived as cost-prohibitive.

7.5. Cardiovascular Magnetic Resonance (CMR)

Cardiovascular magnetic resonance delineates precisely the different tissue components in acute myocardial infarction such as necrosis, microvascular obstruction (MVO), haemorrhage, and oedema, i.e. areas at risk. Cardiovascular magnetic resonance has emerged as a powerful technique to assess myocardial ischaemia and viability. This is an ideal non-invasive imaging modality in women. Cardiac MRI can be used to diagnose vasospasm. Currently, cardiac MRI techniques are available to acquire images that can assess morphology, function, perfusion, coronary vascular dysfunction and viability.

Stress CMR imaging uniquely allows measurement of subendocardial perfusion. In an initial report in symptomatic women with abnormal stress tests and normal coronaries, subendocardial ischemia was frequently observed. A strong correlation between subendocardial ischemia and abnormal coronary reactivity testing has been reported in women. [44].

The ability to non-invasively assess coronary vasoreactivity would be particularly important in the diagnosis and treatment of women as an early marker of subclinical atherosclerosis [45].

7.6. Coronary CT for Coronary Artery Calcium (CAC)

Coronary CT detects and quantifies the amount of coronary artery calcium (CAC), a marker of atherosclerotic disease burden, via either electron beam tomography (EBT) or multidetector CT (MDCT). CAC scores approximate the total atherosclerotic plaque burden and also has a prognostic value. Women have less prevalent and less severe CAC than do men. [40]

Although not specific for luminal obstruction, the CAC score provides an estimate of the total atherosclerotic plaque burden and thereby provides information regarding cardiac risk. Sex-specific data on the role of CAC in evaluating at-risk symptomatic women demonstrate a high negative predictive value when correlated with coronary angiography.

7.7. Cardiac CT Angiography (CTA)

The introduction of updated software and 64-slice multidetector technology facilitates the noninvasive anatomic assessment of obstructive CAD with a high diagnostic accuracy with sensitivities and specificities in the ranges of 80% to 98% and 86% to 98%, respectively. No sex-specific differences were noted with contemporary computed tomographic angiography. [46]

7.8. Role of Cardiac Biomarkers

In patients with Acute Coronary Syndromes, specifically, men are more likely to have elevated creatine kinase MB and troponins, whereas women are more likely to have elevated C-reactive protein (hs CRP) and Brain Natriuretic Peptide (BNP).[47] Inflammation is believed to have a more important role in the pathogenesis of cardiovascular events in females. Therefore, the measurement of inflammation markers has been proposed as a method to predict these events particularly in women. Newer markers are Apolipoprotein E polymorphism and serum amyloid A. [47].

7.9. Coronary Intravascular Ultrasound

Unlike angiography, which depicts a silhouette of the coronary lumen, intravascular ultrasound portrays the vessel from a tomographic, cross-sectional perspective. This orientation enables direct measurements of luminal dimensions, including minimum and maximum diameter and cross-sectional area. The ability of coronary ultrasound to image the soft tissues within the arterial wall enables characterization of atheroma size, plaque distribution, and lesion composition during diagnostic or therapeutic catheterization.

7.10. Recommended Diagnostic Strategy for Evaluating CAD in Women

In women with a history of angina as has been described and risk factors discussed ECG is to be done first although it is often normal. Exercise testing is done in women who are able to exercise and have intermediate risk. The functional capacity, and clinical scores such as the Duke treadmill score provide additional information about the likelihood of CAD. A normal ECG or even a negative TMT should not be ignored in presence of a conventional risk factor and the history of angina and further investigations should be done.

Both the diagnostic accuracy and prognostic value of stress SPECT and stress echocardiography exceed those of exercise ECG alone. Combining the two tests for example exercise stress testing in combination with SPECT may reduce the false negative rate. American Heart Association now recommends the use of Stress Echocardiography or SPECT as an initial test for evaluation of suspected ischemic heart disease in women with symptoms and who also additionally have diabetes, functional impairment (i.e., who cannot achieve at least 5 METs of exercise on a treadmill or stationary bicycle) or an abnormal resting ECG eg a bundle branch block which does not allow proper interpretation of ST-segment depression with exercise .Stress MPI / Stress Echocardiography provide useful information in women with suspected or known CAD. Stress-induced regional wall-motion abnormalities and myocardial perfusion defects have relatively similar sensitivities and specificities for IHD in women. The use of either imaging modality is appropriate for either gender, and the choice of test should depend on the experience and cost at individual institutions [48]

In investigating CAD, the issue of gender difference in the referral pattern for coronary angiography is controversial. In women older than 49 who have undiagnosed chest pain, coronary angiography may be necessary for the diagnosis of CAD when either the exercise stress test (EST) or the stress imaging study (SIS) is positive. In contrast, in women age 49 or younger who have chest pain of uncertain origin, the use of angiography to rule out CAD may be regarded as necessary only if both the EST and the SIS are positive. Recently, it has been suggested that, because noninvasive tests are less sensitive and specific for diagnosing CAD in women, the threshold for coronary angiography in women should be lowered. If vasospastic disease is suspected, coronary angiography may be appropriate. For unstable angina or perimyocardial infarction, coronary angiography may be indicated.

Cardiac CTA may be done if the stress imaging is abnormal to evaluate coronary arteries non-invasively. Patients with decreased cardiac function may be evaluated by invasive coronary angiography.[43] CMR can be useful in evaluating symptomatic women with no evidence of obstructive CAD to evaluate the coronary microvasculature for evidence of subendocardial ischemia or abnormal coronary reserve. Coronary CT can provide additional information.

Measurements of Cardiac Biomarkers for decision making in women with ACS are at least as essential as in men. Troponins identify the group of patients who benefit from an aggressive approach similarly to men and, in addition and just as important, point out female patients with negative troponin results in whom aggressive treatment is not required.

Other investigations can be done according to the availability e.g. PET scan. Wherever available patients with non-diagnostic or equivocal stress echo /nuclear testing who were tested for ischemic symptoms, who cannot exercise , who are obese and with large pendulous breasts and need pharmacologic testing , high CAC , Coronary CTA showing borderline obstructive plaque, and patients requiring a decision regarding the need for revascularization, either from the ischemic or viability standpoint, would qualify for a PET scan. Of the tests available to demonstrate coronary spasm, provocation by methylergonovine maleate, acetylcholine, or hyperventilation is the most useful.

8. Social Factors Pertaining to CAD in Women

Changing lifestyles are being blamed for the sharp increase in CAD in women. More women now work in stressful careers where activity levels are low. These women often have unhealthy eating habits and indulge in excessive smoking and drinking. Busy housewives and mothers also face heart risks because often they don't have time to exercise and eat junk food.

Women tend to minimize their symptoms and have poorer psychosocial adjustment following a CAD event. They are less likely to receive aspirin, beta-blockers, statins, antiarrhythmic treatment, thrombolytic therapy, Cardiac Resynchronisation Therapy (CRT), an implantable cardioverter defibrillator (AICD) and cardiac transplant strategies.[49] However the situation has improved somewhat in the recent years in the developed countries. In fact the major contribution to the increased survival among women since 2000 appears to be improved care of established cardiovascular disease rather than a decrease in the occurrence of new cases of cardiovascular disease in women, emphasizing the need for preventive interventions.

This does not hold true for the developing countries like India where not much medical improvements have occurred. Advanced investigations are not available especially to the poorer section. India and many other countries of the world are male dominated societies where females have lesser treatment resources. They generally receive suboptimal and less-aggressive therapy following cardiac ischemic events. Women are less likely to have an ECG when they have an episode of ischemic heart disease or chest pain and are much less likely to be submitted to sophisticated diagnostic facilities and treatment modalities like coronary angiography, PTCA /CABG as compared to men. In Indian women obesity is increasing with increasing incidence of coronary artery disease. Coronary artery disease and coronary risk factors were two or three times higher among the urban compared with the rural subjects.[50] It is possible that some Indian populations especially women can benefit by reducing serum cholesterol, blood pressure and central obesity and increasing physical activity.

9. Reasons for the Existing Gender Differences

The complete explanation for the unfavorable prognosis of women after acute coronary events and myocardial infarction is far from elucidated. Is it a bias in patient-care patterns? Or is it a different disease with a more prominent microvascular/ inflammatory component that provokes symptoms of ischemia without obstructive coronary artery disease.

It is possible that unaccounted comorbidities and risk factors are responsible for the outcome differences. Alternatively, other unknown factors may be involved; among these, social and psychological factors have rarely been considered. A recent study, for example documented a remarkable decrease in mortality in women with coronary heart disease randomly assigned to a stress-reduction intervention specifically tailored to women [51].

Possible mechanisms for poorer outcomes in women are lack of awareness and differences in access to treatment, smaller vessels and lesser

collateralization and impaired vasomotor response and the unique pathophysiology.

10. Treatment

Optimal treatment for CAD in women is not as well tailored as in men, but a multi-pronged approach seems to be best, and should include aggressive risk factor modification, therapy to reduce the risk of clotting (aspirin) and drugs to protect the heart muscle itself (beta blockers and possibly ACE inhibitors) and statins to improve lipid profile. [52] They have about a 50% higher bleeding rate than men do after adding antiplatelet agents, heparin or Gp IIb/IIIa inhibitors. For women, hypertension and diabetes need to be treated more aggressively with greater attention to increased physical activity & healthy lifestyle. A thorough assessment should be made for deciding specialised PCI/ CABG procedure taking into consideration the specific challenges pertaining to female gender & keeping in mind the other comorbid conditions. Previous studies have suggested that women did not do as well with bypass surgery as compared to men. And much of this had to do with smaller body habitus, coronary artery size and functional status and numerous risk factors including diabetes. An encouraging fact is that in-hospital mortality rates are declining in women and CABG outcomes are improving .It is likely that technical advancements in surgical and myocardial protection techniques, such as the increasing use of off-pump procedures, the increasing use of internal mammary artery grafts and the ability to successfully operate on small arteries, have contributed and will continue to contribute to the narrowing of the gender gap in the risks and benefits of CABG surgery.[53]

More and more females should be submitted to post MI and post CABG rehabilitation programmes. The 30-day mortality after PCI in women has also decreased in the past 25 years. After accounting for baseline risks, no differences in short-term or long-term mortality were observed between men and women [54].

11. Role of Hormone Replacement Therapy (HRT)

Oestrogen helps in cardioprotection by improving endothelial function and by decreasing inflammation in the vessel wall. Oestrogen deficiency increases the progression of atherosclerosis. Initial observational studies in late eighties and early ninieties had shown role of HRT in women for protection against CAD and its complications .However the subsequent randomized controlled trials like WHI have shown that instead of cardioprotection, HRT may be causing more CAD & its sequelle on long term use especially in late onset cases.

The suggestion from the Women's Health Initiative study that estrogen intervention at or shortly after the menopause may be protective now known as the “critical timing” hypothesis warrants further investigation. [55] At present HRT cannot be routinely recommended for cardioprotection against CAD in women.

Conclusion

Coronary artery disease in women is an important health problem. But unfortunately it is less recognized than in men due to the atypical symptoms and gender bias in society especially in developing countries like India .In the past few decades there has been growing awareness regarding the peculiarities of coronary artery disease in women. In women, the detection and evaluation of physiologically significant CAD is challenging, especially given that traditional tests designed to detect focal areas of coronary artery stenosis are less sensitive and specific .More research is needed in the areas of sex-specific aspects of coronary artery disease and large clinical trials are needed to explore these sex-related differences so that optimal gender specific diagnostic and management strategies can be developed for females. More health education and public awareness programmes including lifestyle modifications and other gender specific issues may go a long way in controlling this major public health problem in the fair sex.

References

[1] Vaccarino V, Parsons L, Peterson ED, Rogers WJ, Kiefe CI, Canto J. Sex differences in mortality after acute myocardial infarction: changes from 1994 to 2006. *Arch Intern Med* 2009; 169:1767–1774.

[2] Berger J, et al. "Sex differences in mortality following acute coronary syndromes," JAMA 2009; 302:874-882

[3] Hochman JS, Tamis JE, Thompson TD et al. Sex, clinical presentation,and outcome in patients with acute coronary syndromes. Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes IIb Investigators. *N Engl J Med* 1999;341:226–32.

[4] Roger VL, Go AS, Lloyd-Jones DM et al. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation* 2011; 123:e18–e209.

[5] Wenger NK. Clinical characteristics of coronary heart disease in women: emphasis on gender differences. *Cardiovascular Research* 2002; 53 558–567.

[6] Ni H, Coady S, Rosamond W et al. Trends from 1987 to 2004 in sudden death due to coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study.*Am Heart J*. 2009;157:46–52.

[7] Bairey merz, CN Shaw LJ, Reis SE et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part II: gender differences in presentation, diagnosis, and outcome with regard to gender based pathophysiology of atherosclerosis and macrovascular and microvascular coronary disease. *J Am Coll Cardiol* 2006 ; 47(3suppl):S21–S29.

[8] Bugiardini R, Merz CN Bairey. Angina with "normal" coronary arteries: A changing philosophy. JAMA 2005; 293:477–484.

[9] Chokshi NP, Iqbal SN, Berger RL et al. Sex and race are associated with the absence of epicardial coronary artery obstructive disease at angiography in patients with acute coronary syndromes. Clin Cardiol 2010;(33):495-501.

[10] Buchthal SD, den Hollander JA, Bairey Merz CN et al. Abnormal myocardial phosphorus-31 nuclear magnetic resonance spectroscopy in women with chest pain but normal coronary angiograms. *N Engl J Med* 2000 ; 342:829 –35.

[11] Vaccarino V . "Ischemic heart disease in women: many questions, few facts.", *Circ Cardiovasc Qual Outcomes* 2010; 3(2): 111–115.

[12] Sun H, ohri M, Shimokawa H, Usai M, Hrakami L, Takeshita A. Coronary microvascular spasm causes myocardial ischemia in patients with vasospastic angina. *J Am Coll Cardiol* 2002; 39:847–51.

[13] Shaw LJ, Bugiardini R, Merz CNB. Women and ischemic heart disease: evolving knowledge. *J Am Coll Cardiol* 2009; 54: 1561–1575.

[14] Gosai J, Malkin C. J., Grech D. Case Report. The Vanishing Stenosis: ST Elevation Myocardial Infarction and Rhythm Disturbance due to Coronary Artery Spasm—Case Report and Review of the Literature 2010 ; Volume 2010 Article ID 132902.

[15] Kovacs KA , Burggraf GW, and Dewar CL Reversible cardiogenic shock in an angry woman—case report and review of the literature. *Canadian Journal of Cardiology* 1996; 12, no. 7, 689–693.

[16] Nathaniel RS, Barbara AS, Christopher RA, Jonathan SS et al. Women Have Less Severe and Extensive Coronary Atherosclerosis in Fatal Cases of Ischemic Heart Disease *American Heart Journal* 2011;161(4) 681-688.

[17] Vidi V, Rajesh V, Singh PP, Mukherjee JT, Lago RM, Venesy DM, Waxman S, Pyne CT, Piemonte TC, Gossman DE, Nesto RW. *Clinical characteristics of Tako-Tsubo cardiomyopathy. Am J Cardiol* 2009;104:*578-582.*

[18] Pepine CJ. Provoked coronary spasm and acute coronary syndromes. *J Am Coll Cardiol* 2008 ;(52):528-530.

[19] Bairey Merz, Pepine CJ. Syndrome X and Microvascular Coronary Dysfunction. *Circulation* 2011; 124: 1477-1480.

[20] Matsuzawa Y, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Konishi M, Matsubara J,et al . Digital assessment of endothelial function and ischemic heart disease in women. *J Am Coll Cardiol* 2010 ; 55(16):1688-96.

[21] Burke AP, Farb A, Malcolm GT, Liang Y, Smialek J, Virmani R. Effects of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. *Circulation* 1998 ; 97:2110–16.

[22] Shaw LJ, Bugiardini R, Merz CNB) Women and ischemic heart disease: evolving knowledge. *J Am Coll Cardiol* 2009; 54: 1561–1575.

[23] Farb A, Burk AP, Tang AL, Youhui Liang, Poonam Mannan, John Smialek, Renu Virmani.\Coronary Plaque Erosion Without Rupture Into a Lipid Core.A Frequent Cause of Coronary Thrombosis in Sudden Coronary Death. *Circulation* 1996 ; 93*:* 1354-1363.

[24] Kramer MCA, Rittersma SZH, de Winter RJ et al. Relationship of thrombus healing to underlying plaque morphology in sudden coronary death. J Am Coll Cardiol 2009; DOI:10.1016/j.jacc.2009.09.007. Available at: http://content.onlinejacc.org.

[25] Reynolds HR, Srichai MB, Iqbal SN, Slater JN, Mancini GB, Feit F, Pena-Sing I, Axel L, Attubato MJ, Yatskar L, Kalhorn RT, Wood DA, Lobach IV, Hochman JS. Mechanisms of myocardial infarction in women without angiographically obstructive coronary artery disease. *Circulation* 2011 ;124(13):1414-25.

[26] Domenico G. Della R, Carl J. Pepine. What Causes Myocardial Infarction in Women Without Obstructive

Coronary Artery Disease? *Circulation* 2011; 124: 1404-1406.

[27] Nicholls SJ, Wolski K, Sipahi I, Schoenhagen P, Crowe T, Kapadia SR, et al. Rate of progression of coronary atherosclerotic plaque in women. *J Am Coll Cardiol* 2007 ; 49:1546–1551.

[28] Anita V. K, Angela D. , Ruth Falik . Ischemic Heart Disease in Women. *South Med J* 2011; 104(3):200-204.

[29] Halberg F, Kabat HF, Klein P. Chronopharmacology: a therapeutic frontier. *Am J Hosp Pharmacy* 1980; 37: 101-6.

[30] Halberg F. Implications of biologic rhythms for clinical practice.*Hosp Pract* 1977; 12: 139-49.

[31] Halberg F, Barnwell F. Chronobiology A science in tune with rhythms of life. 1986; Published by Earl Bakken 7000 Central Avenue NE Minneapolis, MN 55432.

[32] Mulcahy D, Dakak N, Zalos G, Andrews NP, Proschan M, Waclawiw MA, Schenke WH, Quyyumi AA. Patterns and behavior of transient myocardial ischemia in stable coronary disease are the same in both men and women: a comparative study.J Am Coll Cardiol 1996;(7):1629-36.

[33] Hasdai D, Porter A, Rosengren A, et al. Effect of gender on outcomes of acute coronary syndromes. *J Am Coll Cardiol* 2003; 91: 1466–1468.

[34] Rosengren A, Wallentin L, Gitt A K , Behar S ,Battler A ,Hasdai D . Sex, age, and clinical presentation of acute coronary syndromes. *Eur Heart J* 2004 ; 25 663–670.

[35] Vaccarino V, Krumholz HM, Yarzebski J, Gore JM, Goldberg RJ. Sex differences in 2-year mortality after hospital discharge for myocardial infarction. *Ann Intern Med.* 2001 ;134(3):173-181.

[36] Li Z, Li ZZ, Gao YL, Tao Y, Wang S, Wang Q, Ma CS, DU X. Clinical and coronary angiographic features of young women with acute myocardial infarction Zhonghua Xin Xue Guan Bing Za Zhi. 2012 Mar;40(3):225-30.

[37] Hennekens C., Evans D., Peto R.; Oral contraceptive use, cigarette smoking and myocardial infarction. Br J Fam Plann. 5 1989:66-67.

[38] W L Lee, A M Cheung, D Cape ,B Zinman. Impact of diabetes on coronary artery disease in women and men: a meta-analysis of prospective studies. Diabetes Care 2000; 23: 962.

[39] Angela H, Yvonne T, Vera R, Eva S, Yolande E, Gerard P, Hugo T et al. Red alert for women's heart: the urgent need for more research and knowledge on cardiovascular disease in women Proceedings of the Workshop held in Brussels on Gender Differences in Cardiovascular disease, 29 September 2010. *Eur Heart J* 2011; 32 (11): *1362-1368.*

[40] Mieres JH, Shaw LJ, Arai A Budoff M, Hundley G, Flamm SD, Marwick TH, Mosca L, Patel AR, Redberg RF, Taubert K, Thomas G, Wenger NK (2005) . Role of noninvasive testing in the clinical evaluation of women with suspected coronary artery disease: Consensus statement from the Cardiac Imaging Committee, Council on Clinical Cardiology, and the Cardiovascular Imaging and Intervention Committee, Council on Cardiovascular Radiology and Intervention, American Heart Association. *Circulation.* 2005;111:682–696.

[41] Alexander KP, Shaw LJ, Shaw LK, Delong ER, Mark DB, Peterson ED.Value of exercise treadmill testing in women. *J Am Coll Cardiol* 1999 ; 32: 1657–1664.

[42] Lerman A , Sopko G. Women and Cardiovascular Heart Disease:Clinical Implications From the Women's Ischemia Syndrome Evaluation (WISE) Study Are We Smarter? *Journal of the American College of Cardiology* 2006; Vol. 47, No. 3 Suppl S by the American College of Cardiology Foundation.

[43] Phillips LM, Mieres J .Noninvasive Assessment of Coronary Artery Disease in Women: What's Next? *Curr Cardiol Rep* 2010 ; 2(2): 147–154.

[44] Pilz G, Klos M, Ali E, Hoefling B, Scheck R, Bernhardt P.Angiographic correlations of patients with small vessel disease diagnosed by adenosine-stress cardiac magnetic resonance imaging. *J Cardiovasc Magn Reson* 2008 ;10(1):8.

[45] Hirsch GM . Non-Invasive Measurement of Coronary Vasoreactiviy using 3T MRI.JCMR 2007; 9: Abstract 597.

[46] Pundziute G, Schuijf JD, Jukema JW, et al. Gender influence on the diagnostic accuracy of 64-slice multislice computed tomography coronary angiography for detection of obstructive coronary artery disease. *Heart* 2008 ;94:48–52.

[47] Wiviott SD, Cannon CP, Morrow DA et al. Differential expression of cardiac biomarkers by gender in patients with unstable angina/non-ST elevation myocardial infarction. A TACTICS-TIMI 18 (Treat Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis in Myocardial Infarction 18) substudy. *Circulation* 2004 ; 109 580-586.

[48] Priya K, Martha G Exercise Stress Testing in Women Going Back to the Basics *Circulation* 2010 ; 122: 2570-2580.

[49] Enriquez JR, Pratap P, Zbilut JP, Calvin JE, Volgman AS Women tolerate drug therapy for coronary artery disease as well as men do, but are treated less frequently with aspirin, beta-blockers, or statins. *Gend Med* 2008; 1 53-61.

[50] R. B. Singh, J. P. Sharma, V. Rastogi, R. S. Raghuvanshi, M. Moshiri, S. P. Verma, and E. D. Janus. Prevalence of coronary artery disease and coronary risk factors in rural and urban populations of north India. European Heart Journal 1997; 18, 1728- 1735.

[51] Orth-Gomer K, Schneiderman N, Wang H-X, Walldin C, Blom M, Jernberg T. Stress reduction prolongs life in Women with coronary disease: the Stockholm Women's

Intervention Trial for Coronary Heart Disease (SWITCHD). *Circ Cardiovasc Qual Outcomes* 2009; 2 25–32.

[52] Lori M, Emelia J. Benjamin, Kathy Berra, Judy L.et alEffectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—Update. *J Am Coll Cardiol* 2011; 57 1404-1423.

[53] O'Rourke DJ, Malenka DJ, Olmstead EM, Quinton HB, Sanders JHJ, Lahey SJ et al. Improved in-hospital mortality in women undergoing coronary artery bypass grafting. Northern New England Cardiovascular Disease Study Group. *Ann Thorac Surg* 2001 ; 71:507–511.

[54] Mandeep Singh, Charanjit S, Bernard J. Gersh, Veronique L. Roger, Malcolm R. Bell, Ryan J. Lennon, Amir Lerman, David R. Holmes J. Mortality Differences Between Men and Women After Percutaneous Coronary Interventions. *J Am Coll Cardiol*.2008; 51(24):2313-232.

[55] Hsia J, Langer RD, Manson JE , Kuller L et al. Conjugated equine estrogens and coronary heart disease: The Women's Health Initiative. *Archives of Internal Medicine* 2006 ;166 (3) 357–365.

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Peripartum Cardiomyopathy

N. S. Neki[1,*], Ranjana Singh[2], Viola Vargova[3], and Viola Mechirova[3]
[1]Govt. Medical College and Guru Nanak Dev Hospital, Amritsar, 143001, Punjab
[2]King George Medical University, Lucknow, India
[3]PJ Safaric University, Kosice, Slovakia

Abstract

Peripartum cardiomyopathy (PPCM) is rare disorder of unknown etiology. It causes significant mortality. Proposed mechanisms of PPCM are prolactin mediated, myocarditis, autoimmunity, genetic, nutritional. Diagnosis is based on clinical and echocardiographic findings. Subsequent pregnancy should be avoided, because it increases chances of relapse. Newer treatment modalities include bromocriptine, cabergolin, pentoxyfylline, intravenous immunoglobulin, showing promising results. Heart transplantation is last option for treatment of PPCM.

Keywords: Peripartum cardiomyopathy (PPCM); Prolactin; Pregnancy induced cardiomyopathy

Introduction

Peripartum cardiomyopathy (PPCM) is a rare disorder, but serious complication of pregnancy. The European Society of Cardiology [1] recently defined peripartum cardiomyopathy as a form of dilated cardiomyopathy that presents with signs of heart failure in the last month of pregnancy or within 5 months of delivery. Diagnosis requires objective evidence of left ventricular (LV) dysfunction with no other explanation for heart failure signs and symptoms [2,3]. The incidence of PPCM in India was 1 in 1374 live births. It is high compared to United States where 1: 3000 live births [4]. But it is low compared to South Africa (1:1000) [5]. In developing countries, incidence is high which may be due to diagnosis made only clinical as well as variations in local cultural, puerperal practice, ecological factors and environmental influence [6]. The etiology of PPCM remains unclear, contributing to the poor outcomes seen in women affected by the disorder, as targeted treatment is not yet available.

* Correspondence: Professor of Medicine, Govt. Medical College and Guru Nanak Dev Hospital, Amritsar, 143001, Punjab, India.,email: drneki123@gmail.com

Etiology

Risk factors are maternal age >30 years, twin pregnancies and history of hypertension, preeclampsia, eclampsia, prolonged use of tocolytics [4,7].

The etiology of peripartum cardiomyopathy remains unknown despite much investigation that has focused on identifying a cause. The patho-physiological mechanisms are still not clarified, and proposed etiology includes viral myocarditis, low selenium levels, stress-activated cytokines, inflammation and autoimmune reaction and a pathologic response to hemodynamic stress [8,9]. Recently studies suggested possibility of a common pathway, on which different etiologies that induce PPCM could merge [10]. This proposed pathway points to a coincidental situation where unbalanced oxidative stress and high levels of the nursing hormone prolactin (PRL) are present [10]. As a result the oxidative stress triggers the activation of the protease cathepsin D that cleaves PRL into an angiostatic and proapoptotic 16-kDa PRL that seems to initiate and drive the disease. The 16-kDa PRL mainly impacts on the endothelium [11-14] with potential subsequent negative effects on cardiomyocyte function [10]. Furthermore, a key functional role of an activated oxidative stress–cathepsin D–16 kDa prolactin cascade in PPCM is strongly supported by the observation that suppression of the production of prolactin by the dopamine D2 receptor agonist, bromocriptine, prevented the onset of PPCM in the mouse model of PPCM [10].

Inflammation

Reports of high concentrations of tumor necrosis factor- α (TNF α), interferon-ϫ, interleukin-6, C‑reactive protein (CRP), and Fas/apoptosis antigen 1 (Apo-1) in peripartum cardiomyopathy suggest an underlying inflammatory process for the pathophysiologic development of peripartum cardiomyopathy. But anti-inflammatory agent pentoxyfylline does not produce clinical benefit in patients with PPCM [15]. James D et al in their study [16] has shown the beneficial effect of pentoxyfylline.

Viral Infections

PPCM is associated with various viral infections like coxackievirus group B and Epstein-Barr virus [17]. Human immunodeficiency virus (HIV) infection is also increasingly being recognized as an important cause of dilated cardiomyopathy (DCM) [18].

Auto Immunity

Lamparter et al found that presence of circulating auto-antibodies to every type of cardiac tissue were identified in all 10 cases screened [19]. Warraich et al reported, in PPCM higher titres of antibodies (IgG and IgG subclasses) against cardiac myosin heavy chain compared with those with IDCM and these titres correlated with clinical presentation and with New York Heart Association (NYHA) functional class [20]. In addition, the potential role of microchimerism, due to the introduction of foetal cells of haematopoietic origin into the maternal circulation, has been raised [21].

Genetic Susceptibility to Peripartum Cardiomyopathy

Recently, however, there have been two reports which more strongly support the suggestion that some cases of PPCM may in fact be part of familial DCM [22, 23]. Other potential causes of PPCM include nutritional deficiencies, coronary artery spasm, small vessel disease and defective antioxidant defences [24].

Pathological features

Heart specimens appear pale, soft, dilated and heavier in PPCM [25]. Due to persistent ventricular dysfunction, mural thrombi are invariably seen in one or more cardiac chambers. Gray-white patches of endocardial thickening are often seen at the sites of mural thrombi [26]. Cardiac valves and coronary vessels appear normal with the occasional presence of pericardial effusion [22]. Histological evidence of hypertrophy, degeneration, fibrosis, interstitial edema,

fatty and mononuclear cell infiltration is seen in the myocardium with a sparse to abundant collection of eosinophils [26]. Electron microscopy has revealed varying degrees of enlargement, destruction or fragmentation of myofibrils, an increase in size and number of mitochondria, glycogen and some abnormal pertinacious material deposits [26]. Histochemical pictures of myocardial cells denote occasional sarcoplasmic fat vacuoles containing triglycerides without any accumulation of lipofuscin or amyloid. Significantly low levels of plasma albumin, prealbumin, selenium and zinc have also been reported [7].

Clinical presentation

Dyspnea on exertion, cough, orthopnea, hemoptysis, and paroxysmal nocturnal dyspnea are commonly seen in patients with PPCM & mimic like left ventricular failure. Most of the patients have New York Heart Association (NYHA) class III or IV function [27]. But use of NYHA classification may not reflect severity, because of normal occurrence of these symptoms in advanced pregnancy. Other nonspecific symptoms include fatigue, malaise, palpitations and postural hypotension. They may present with embolic features like chest pain, abdominal discomfort, hemoptysis and hemiplegia [27].

Diagnosis requires a high degree of suspicion, because symptoms of peripartum cardiomyopathy can be confused with physiologic changes associated with advanced pregnancy. Common signs of peripartum cardiomyopathy include displacement of the apical impulse, presence of S3, and evidence of mitral or tricuspid regurgitation. Engorgement of the neck veins, pulmonary crepitations, hepatomegaly, and pedal edema may also be present. Patients may even present with seizures associated with cerebral edema and cerebellar herniation [27,28].

Investigations

Blood tests should be done in all patients, although none of these can help in screening or positive diagnosis of PPCM. Initial laboratory assessment should include complete blood count and biochemical parameters. The thyroid function, a septic screen, and viral serology should also be performed in order to exclude other causes of cardiomyopathy and heart failure.

ECG

No pathognomic changes in electrocardiograms have been identified for peripartum cardiomyopathy. ECG abnormalities are sinus tachycardia, nonspecific ST-T segment changes, LV hypertrophy, premature ventricular contractions, and bundle branch block [29]. However, these changes may be present in a number of disorders as well as normal states. Sometimes the ECG may demonstrate no significant changes [30].

Doppler Echocardiography

Doppler echocardiography is useful diagnostic tool for assessing the severity and prognosis of PPCM patients. Echocardiographic features include decreased ejection fraction (<45%), decreased left ventricular fractional shortening (<30%) and increased left ventricular end diastolic diameter (>2.7cm/m^2) [31]. Other features are dilatation of all cardiac chambers, mitral, tricuspid, pulmonary and aortic regurgitation, diffuse wall motion abnormality and small pericardial effusions are also reported [27]. LV thrombus is present at diagnosis in 17% of patients and indicates poor prognosis [32]. MRI is a more sensitive tool to diagnose such thrombi than echocardiography. Echocardiographic measurements have been used for prognostication of PPCM but dobutamine stress echocardiography, having the capability to show the contractile reserve, may be a better tool in this regard & can help identify those at risk of relapse [33].

Chest Radiographs

Chest radiographs should be obtained in suspected cases of PPCM [34]. They may be helpful to identify acute pulmonary edema. Radiological

indications of heart failure such as cardiomegaly, pulmonary congestion and pleural effusions may be evident [34]. However, diagnosing cardiomegaly on the basis of a chest radiograph in a pregnant patient is difficult because the heart is pushed upward and laterally, giving the false impression of cardiomegaly.

Cardiac Biomarkers

In the initial evaluation, the serum level of troponin may be helpful in ruling out myocardial infarction, however an increase in troponin in the acute phase of PPCM, without myocardial infarction can occur. Levels of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide can help in confirming the diagnosis [35,36].

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) may be used as a complementary tool to diagnose peripartum cardiomyopathy, and it may prove to be important in identifying the mechanisms involved. It can measure global and segmental myocardial contraction, and it can characterize the myocardium [37]. Delayed contrast enhancement (with gadolinium) can help to differentiate the type of myocyte necrosis, i.e. myocarditis vs. ischemia. Myocarditis has a nonvascular distribution in the subepicardium with a nodular or band-like pattern, whereas ischemia has a vascular distribution in a subendocardial or transmural location [38]. Recently Cardiac Magnetic Resonance Imaging (CMRI) has been used in prognostication in PPCM. Leurent et al advocate using cardiac MRI to guide biopsy to the abnormal area, which may be much more useful than blind biopsy [39].

Endomyocardial Biopsy

Endomyocardial biopsy is a highly specific technique for diagnosing myocarditis [34]. However, its invasive nature, coupled with the varying incidence of myocarditis in peripartum cardiomyopathy, precludes its use as a first-line diagnostic tool [27]. Biopsy under MRI guidance improves accuracy and can be followed up with polymerase chain reaction analysis of biopsy DNA extracts for viral assays or immunohistochemical staining for autoantibody assays. Endomyocardial biopsy might be considered when myocarditis is strongly suspected or no improvement is seen after 2 weeks of heart-failure therapy [27].

Self-test for Early Diagnosis of Heart Failure in PPCM

Fett proposed a screening tool for early diagnosis of PPCM. The test is a focused medical history for PPCM screening, looking for the most common early signs and symptoms of heart failure during last month of pregnancy [33]. He proposes 6 clinical categories, easy to quantify, which are included in a self-scoring system (Table 1).

Table 1. Self-test for early diagnosis of heart failure in PPCM (adapted from Fett JD) [30]

Sign/symptom	Characteristics	Scoring
Orthopnea	None	0
	Need to elevate head	1
	Need to elevate ≥ 450	2
Dyspnea	None	0
	Climbing 8 or more steps	1
	Walking on level	2
Unexplained cough	None	0
	At night	1
	Day and night	2
Swelling lower extremities	None	0
	Below knee	1
	Above and below knee	2

Sign/symptom	Characteristics	Scoring
Excessive weight gain (during last month of pregnancy)	< 2 pounds/week 2-4 pounds/week > 4 pounds per week	0 1 2
Palpitations	None When lying down at night Day and night, any position	0 1 2

Scoring and action
0-2 = low risk – continue observation
3-4 = mild risk – consider doing blood BNP and CRP; echocardiogram if BNP and CRP are elevated
5 or more = high risk – do blood BNP, CRP and echocardiogram

A score ≥ 5 has always been associated with LV systolic dysfunction. A score > 4 suggests the need for further investigation. In this case, a blood BNP test and an echocardiography are recommended. If the score is < 4, the patient should be monitored for BNP and C-reactive protein levels. If increased levels, echocardiography should be performed. Fett proposed that this test is not diagnostic for PPCM, but encourages an expanded use, because it may be a useful tool for early recognition of the new onset heart failure [33].

Management

Heart failure treatment during pregnancy

When considering tests or treatments in pregnancy, the welfare of the fetus is always considered along with that of the mother.

Angiotensin-converting enzyme-inhibitors and angiotensin-II receptor blockers

Angiotensin-converting enzyme (ACE)-inhibitors and angiotensin-II receptor blocker (ARB) are contraindicated because of serious renal and other foetal toxicity [40].

Hydralazine and long-acting nitrates

It is believed that this combination can be used safely, instead of ACE-inhibitors/ARBs, in patients with PPCM [41].

Beta blockers

These have not been shown to have teratogenic effects [41]. Beta-1selective drugs are preferred because beta-2 receptor blockade can, theoretically, have an anti-tocolytic action.

Diuretics

Diuretics should be used sparingly as they can cause decreased placental blood flow [43]. Furosemide and hydrochlorothiazide are most frequently used.

Aldosterone antagonists

Spironolactone is thought to have antiandrogenic effects in the first trimester [44]. Because the effects of eplerenone on the human foetus are uncertain, it should also be avoided during pregnancy.

During Post-Partum period- Treatment is identical to that for nonpregnant women with dilated cardiomyopathy. ACE inhibitors and ARBs are useful.

Antiarrhythmic agents

Antiarrhythmic agents may sometimes be required to treat symptomatic patients. No antiarrhythmic agent is completely safe in pregnancy. Quinidine and Procainamide should be tried first because of their higher safety profile [45]. Treatment should always start in a hospital setting because of the high incidence of torsades de pointes associated with their use. Beta blockers can also be used [45,46]. Digoxin can be considered for atrial arrhythmias, and adenosine can also be used in an emergency [46]. However, class 3 (amiodarone) and class 4 (verapamil) antiarrhythmics should be avoided as a result of adverse effects on the fetus: fetal hypothyroidism and premature delivery, fetal bradycardia, heart block, and hypotension. Amiodarone may cause hypothyroidism, growth retardation and perinatal death, so it should be

avoided in the first trimester and reserved for life threatening arrhythmias only [45]. Though primarily indicated in bradyarrhythmia, pacemakers have been used in PPCM patients for refractory arrhythmias [45]. Automated implantable cardiverter – defibrillator (AICD) can be considered in life threatening arrhythmias and has been shown to reduce the risk of sudden death in PPCM patients [45].

Anticoagulation

Pregnancy is hypercoagulable state. In PPCM due to ventricular dysfunction stasis occurs which leads to thrombus formation. This state extends upto 6 weeks after delivery. Indications for anticoagulations are LVEF <35%, presence of atrial fibrillation, mural thrombi, obese patients and those with history of thromboembolism. Heparin can be given during pregnancy & after delivery. Warfarin is teratogenic and must be avoided in antepartum [47].

Immunosuppression

Role for immunosuppressive therapy in the treatment of PPCM is based on evidence that PPCM is the result of myocarditis [48]. Presently there is no indication for routine immunosuppressive therapy, but it can be considered in biopsy-proven PPCM patients who do not respond to standard medical management after two weeks [48].

Bromocriptine and Cabergoline

Recent studies have suggested the role of prolactin breakdown products in the aetiology of PPCM. Jahns et al. [49] stated that bromocriptine, a dopamine antagonist that inhibits prolactin secretion, prevented the expected deterioration in the size of the left ventricle and systolic function when given in addition to standard heart failure therapy in a woman with PPCM. De Jong et al. [50] argue that the benefit of using cabergoline, another potent dopamine receptor antagonist like bromocriptine, is the long half-life, 14 to 21 days, of cabergoline, so a single dose is often enough. It seems to be most important modality of treatment, but it needs further studies to prove it.

Pentoxifylline

Treatment with pentoxifylline, a xanthine derived agent known to inhibit the production of tumour necrosis factor alpha, has been shown to improve functional class and left ventricular function in patients with idiopathic dilated cardiomyopathy. Similar to other etiologies of left ventricular dysfunction, elevated levels of TNF alpha have been found in these patients [15].

Immunoglobulin therapy

Human intravenous immunoglobulin (IVIG) has been shown to improve ventricular dysfunction in six PPCM patients, although it failed to show any advantage in IDCM patients. Considering the increasing evidence of autoimmunity in PPCM, it may be prudent to consider IVIG in PPCM patients who do not respond to conventional treatment [51].

Plasmapharesis has also been utilized effectively for this purpose, and may be an alternative to immune globulin therapy in peripartum cardiomyopathy.

Interventions

Patient with PPCM has persistently severe LV dysfunction 6 months following presentation, despite optimal medical therapy, many clinicians would advise implantation of an ICD (combined with CRT if the patient has NYHA functional class III or IV symptoms and a QRS duration 120 ms). Extracorporeal membrane oxygenation has been tried successfully in some patients as a bridge to recovery [52]. Ventricular tachycardia leading to cardiac arrest has been reported in PPCM patients, to avoid such situation increasing use of automated implantable cardioverter defibrillator (AICD) is being tried [53,54].

Cardiac transplantation

Two reports comparing results of cardiac transplantation in age matched females with peripartum cardiomyopathy and idiopathic cardiomyopathy showed favourable and comparable long term survival in both the groups [55,56].

Prognosis

Sliwa et al. [15] found that ejection fraction was the strongest predictor of outcome in women with PPCM. Half of the women will normalize their ejection fraction during follow-up within six months. Women whose left ventricular function does not

recover, prognosis remains guarded and mortality rates as high as 10-50% have been reported [57]. Mrbidities include LV heart failure, persistent left ventricular dysfunction (LVEF<45% after six months of PPCM), increased risk of thromboembolism, preterm labour and its neonatal consequences etc. [31]. One of the most frequently cited issues for women who survive PPCM is whether or not they can safely become pregnant again. No clearly established recommendations for future pregnancies in these women exist [41]. Left ventricular recovery and function are considered the most reliable prognostic factors and predictors of survival in subsequent pregnancies [41]. Future pregnancies are not recommended in women with persistent heart failure, because the heart most likely would not be able to tolerate the increased cardiovascular workload associated with the pregnancy [58]. The subset of women with persistent left ventricular systolic dysfunction should be counselled against subsequent pregnancies; the risks are 19% higher for maternal death than among women with PPCM whose heart failure has resolved [33].

Conclusion

Peripartum cardiomyopathy is a rare but serious condition of unknown cause that affects childbearing women. High degree of suspicion is necessary for diagnosis of PPCM, because earlier diagnosis and treatment improve survival of the patient. Treatment includes standard heartfailure & disease specific targeted therapy including bromocriptin, cabergoiln, pentoxyfylline. Further studies are needed for incidence and to find out specific etiology & treatment.

References

[1] Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2008; 29:270–276.

[2] Pearson GD, Veille JC, Rahimtoola S et al. Peripartum cardiomyopathy. National Heart, Lung, and Blood Institute and Office of Rare Diseases (National Institutes of Health) workshop recommendations and review. *Journal of the American Medical Association* 2000; 283:1183-1188.

[3] Hibbard JU, Lindheimer M, and Lang RM. A modified definition for peripartum cardiomyopathy and prognosis based on echocardiography. *Obstetrics and Gynecology* 1999; 94: 311-316.

[4] Mielniczuk LM, Williams K, Davis DR et al. Frequency of peripartum cardiomyopathy. *American Journal of Cardiology* 2006; 97:1765-1768.

[5] Desai D, Moodley J, Naidoo D. Peripartum cardiomyopathy: Experiences at King Edward VIII Hospital, Durban, South Africa and a review of the literature. *Trop Doct* 1995:25:118-123.

[6] Veille JC, Zaccaro D. Peripartum cardiomyopathy: Summary of international survey on peripartum cardiomyopathy. *Am J Obstet Gynecol* 1999; 181(2): 315-319.

[7] Heider AL, Kuller JA, StraussRA, et al. Peripartum cardiomyopathy: a review of the literature. *Obstetrical and Gynecological Survey* 1999; 54:526-531.

[8] Brar SS, Khan SS, Sandhu GK, et al. Incidence, mortality, and racial differences in peripartum cardiomyopathy. *Am J Cardiol.* 2007;100:302–4.

[9] Sliwa K, Forster O, Libhaber E, et al. Peripartum cardiomyopathy: inflammatory markers as predictors of outcome in 100 prospectively studied patients. *Eur Heart J*. 2006;27:441–6.

[10] Hilfiker-Kleiner D, Kaminski K, Podewski E, et al. A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. *Cell* 2007;128:589–600.

[11] Gonzalez C, Parra A, Ramirez-Peredo J, et al. Elevated vasoinhibins may contribute to endothelial cell dysfunction and low birth weight in preeclampsia. *Lab Invest.* 2007;87:1009–17.

[12] Tabruyn SP, Nguyen NQ, Cornet AM, et al. The antiangiogenic factor, 16-kDa human prolactin, induces endothelial cell cycle arrest by acting at both the G0-G1 and the G2-M phases. *Mol Endocrinol*. 2005;19:1932–42.

[13] Tabruyn SP, Sabatel C, Nguyen NQ, et al. The angiostatic 16 K human prolactin overcomes endothelial cell anergy and promotes leukocyte infiltration via NF-{kappa}B activation. *Mol Endocrinol* 2007.

[14] Tabruyn SP, Sorlet CM, Rentier-Delrue F, et al. The antiangiogenic factor 16 K human prolactin induces caspase-dependent apoptosis by a mechanism that requires activation of nuclear factor-kappaB. *Mol Endocrinol.* 2003;17:1815–23.

[15] Sliwa K, Skudicky D, Candy G, et al. The addition of pentoxifylline to conventional therapy improves outcome in patients with peripartum cardiomyopathy. *Eur J Heart Fail* 2002;4:305–309.

[16] James D, Fett Herryo Sanon. Pentoxyfylline treatment for peripartum cardiomyopathy. *Journal of Cardiac Failure.* 2013,19(8):65-66.

[17] Rose R, Skopic, Hersh J. Peripartum cardiomyopathy. A case report. *Am J Clin Med* 2004; 1: 20-25.

[18] Barbaro G, Di LG, Grisorio B, Barbarini G. Incidence of dilated cardiomyopathy and detection of myocardial cells of HIV-positive patients. *N Engl J Med* 1998;339:1093-9.

[19] Lamparter S, Pankuweit S, Maisch B. Clinical and immunologic characteristics in peripartum cardiomyopathy. *Int J Cardiol* 2007;118:14–20.

[20] Warraich RS, Sliwa K, Damasceno A, et al. Impact of pregnancy-related heart failure on humoral immunity: clinical relevance of G3-subclass immunoglobulins in peripartum cardiomyopathy. *Am Heart J* 2005;150:263–269.

[21] Sundstrom JB, Fett JD, Carraway RD, et al. Is peripartum cardiomyopathy an organ-specific autoimmune disease? *Autoimmun Rev* 2002;1:73–77.

[22] Spaendonck-Zwarts K, Tintelen J, Veldhuisen DJ, et al. Peripartum cardiomyopathy as part of familial dilated cardiomyopathy. *Circulation* 2010;121:2169–2175.

[23] Morales A, Painter T, Li R, et al. Rare variant mutations in pregnancy-associated or peripartum cardiomyopathy. *Circulation* 2010;121:2176–2182.

[24] Michael MG. Peripartum cardiomyopathy. *Circulation.*2013;127:622-26.

[25] Homans DC. Peripartum cardiomyopathy. *N Engl J Med* 1985;312:1432-7.

[26] O'Connell JB, Costanzo-Nordin MR, Subramanian R, et al. Peripartum cardiomyopathy: clinical, hemodynamic, histologic and prognostic characteristics. *J Am Coll Cardiol* 1986;8:52- 6.

[27] Bhakta P, Biswas BK, Banerjee B. Peripartum cardiomyopathy: review of the literature. *Yonsei Med J* 2007;48(5):731-47.

[28] Lampert MB, Lang RM. Peripartum cardiomyopathy. *Am Heart J* 1995;130(4):860-70.

[29] Adesanya CO, Anjorin FI, Adeoshun IO, et al. Peripartum cardiac failure. A ten year follow-up study. *Tropical and Geographical Medicine* 1989; 42:190-196.

[30] Hilfiker-Kleiner D, Meyer GP, Schieffer E, et al. Recovery from postpartum cardiomyopathy in 2 patients by blocking prolactin release with bromocriptine. *Journal of the American College of Cardiology* 2007; 50: 2354-2355.

[31] Debasmita M, Chaitalli D. Peripartum Cardiomyopathy coexistant with human immunodeficiency virus: *Heart Views.* 2013;14(1):26-28.

[32] Amos AM, Jaber WA, Russell SD. Improved outcomes in peripartum cardiomyopathy with contemporary. *Am Heart J* 2006;152:509-13.

[33] Fett JD, Fristoe KL, Welsh SN. Risk of heart failure relapse in subsequent pregnancy among peripartum cardiomyopathy mothers. *Int J Gynaecol Obstet* 2010;109(1):34-6.

[34] Williams J, Mozurkewich E, Chilimigras J, et al. Critical care in obstetrics: pregnancy-specific conditions. *Best Pract Res Clin Obstet Gynaecol* 2008;22(5):825-846.

[35] Lata I, Gupta R, Sahu S, Singh H. Emergency management of decompensated peripartum cardiomyopathy. *J Emerg Trauma Shock.* 2009;2(2):124-128.

[36] Jessup M, Abraham W, Casey D, et al. 2009 Focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol.* 2009;53(15):1343-1382.

[37] Di Bella G, de Gregorio C, Minutoli F, et al. Early diagnosis of focal myocarditis by cardiac magnetic resonance. *Int J Cardiol* 2007; 117:280–281.

[38] Laissy JP, Hyafil F, Feldman LJ, et al. Differentiating acute myocardial infarction from myocarditis: diagnostic value of early- and delayed-perfusion cardiac MR imaging. *Radiology* 2005; 237:75–82.

[39] Leurent G, Baruteau AE, Larralde A, et al. Contribution of cardiac MRI in the comprehension of peripartum cardiomyopathy pathogenesis. *Int J Cardiol* 2009; 132:e91–e93. Epub 2008 Feb 6.

[40] Cooper WO, Hernandez-Diaz S, Arbogast PG, et al. Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med* 2006;354:2443–2451.

[41] Moioli M, Valenzano Menada M, Bentivoglio G, et al. Peripartum cardiomyopathy. *Arch Gynecol Obstet* 2009;281:183–188.

[42] Ghuman N, Rheiner J, Tendler BE, et al. Hypertension in the postpartum woman: clinical update for the hypertension specialist. *J Clin Hypertens* (Greenwich) 2009;11:726–733.

[43] Egan DJ, Bisanzo MC, Hutson HR. Emergency department evaluation and management of peripartum cardiomyopathy. *J Emerg Med* 2009;36:141–147.

[44] Muldowney JA, Schoenhard JA, Benge CD. The clinical pharmacology of eplerenone. *Expert Opin Drug Metab Toxicol* 2009;5:425–432.

[45] Ardehali H, Kasper EK, Baughman KL. Peripartum cardiomyopathy. Minerva Cardioangiol 2003;51:41-8.

[46] Ravikishore AG, Kaul UA, Sethi KK, et al. Peripartum cardiomyopathy: prognostic variables at initial evaluation. *Int J Cardiol* 1991;32:377-80.

[47] Bates GM, Greer IA, Hirsh J et al. Use of antithrombotic agents during pregnancy; the seventh ACCP conference on Antithrombotic and Thrombolytic therapy. *Chest* 2004;126:627S- 644S.

[48] Mason JW, O'Connell JB, Herskowitz A, et al. A clinical trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. *N Engl J Med* 1995;333(5): 269-75.

[49] Jahns BG, Stein W, Hilfiker-Kleiner D et al; Peripartum cardiomyopathy- a new treatment option by inhibition of prolactin secretion. *Am J Obstet Gynecol* 2008;199; 5-6.

[50] De Jong JS, Rietveld K, van Lochem LT et al; Rapid left ventricular recovery after cabergoline treatment in a patient with peripartum cardiomyopathy. *Eur J Heart Fail* 2009;11:220-2.

[51] Bozkurt B, Villaneuva FS, Holubkov R, et al. Intravenous immune globulin in the therapy of peripartum cardiomyopathy. *J Am Coll Cardiol* 1999;34:177-80.

[52] Palanzo DA, Baer LD, Banayosy A, et al; Successful treatment of peripartum cardiomyopathy with extracorporeal membrane oxygenation. *Perfusion* 2009;24:75-9.

[53] Yahagi N, Kumon K, Nakatani T, et al. Peripartum cardiomyopathy and tachycardia followed by multiple organ failure. *Anesth Analg* 1994;79:581-2.

[54] Elkayam U, Akhter MW Singh H, et al. Pregnancy associated cardiomyopathy: clinical characterstics and a comparison between early and late presentation. *Circulation* 2005;111:2050- 5.

[55] Keogh A, McDonald P, Spratt P, et al. Outcome in peripartum cardiomyopathy after heart transplantation. J *Heart Lung Transplant* 1994;13:202-207.

[56] Peter R, Rickenbacher MD. Long term outcome after heart transplantation for peripartum cardiomyopathy. *Am Heart J* 1994;127:1318-23.

[57] Ro A, Frishman Wh. Peripartum cardiomyopathy. *Cardiol Rev* 2006;14:35-42.

[58] Ramaraj R, Sorrell VL. Peripartum cardiomyopathy: causes, diagnosis, and treatment. *Cleve Clin J Med.* 2009;76(5): 289-296.

World Heart Journal
Volume 6, Number 2

ISSN: 1556-4002

Some Pitfalls in Testing Antiatherogenic Agents in Cell Cultures

Sergei V. Jargin[*,1] and Douglas W. Wilson[2]
[1]Peoples' Friendship University, Moscow, Russian Federation
[2]School of Medicine and Health Sciences, Durham, UK

Abstract

There is a tendency that some placebos and potentially harmful agents are presented as evidence-based medications; while theoretical concepts are sometimes created or existing ones misused for this purpose. In a large series of studies, having become known internationally after a publication in The Lancet (1986;2:595) and continued until the present day, it was reported that culturing of smooth muscle cells or macrophages with the serum from atherosclerosis patients caused intracellular cholesterol accumulation, whereas serum from healthy controls had no such effect. The cell cultures were used for evaluation of antiatherogenic drugs and dietary supplements including natural products; and corresponding scientific articles were used for their official registration. It is however known that antiatherogenic agents can influence lipid metabolism, cholesterol synthesis, or endothelium-related mechanisms. All these targets are absent in the cell monocultures. In vivo, relationship between cholesterol uptake by cells and atherogenesis is inverse rather than direct; for example, in familial hypercholesterolemia and other conditions discussed here, decreased clearance of LDL-cholesterol by cellular uptake causes hypercholesterolemia and predisposes to atherosclerosis. In conclusion, inadequate testing systems are sometimes used for registration of drugs and dietary supplements. As a result, supposedly antiatherogenic medications with unproven effects are then offered to the elderly patients misinformed not only by advertising but also by certain scientific papers.

Keywords: Atherosclerosis treatment; Serum atherogenicity; Cell culture; Placebo

Introduction

Manufacturers of medical products need financial return on their investments. At the same time, there must be a favorable risk-benefit balance for the patient, confirmed according to the evidence-based principle. Patients and physicians can be, and

[*] Correspondence: Dr. Sergei V Jargin, Peoples' Friendship University of Russia, Clementovski per 6-82, 115184 Moscow, Russia. Tel/fax: +7 495 9516788; E-mail: sjargin@mail.ru

sometimes indeed are, influenced by advertising, which can be facilitated by gaps in education or by hidden adverts in peer-reviewed journals in the guise of the medico-scientific literature. Worrisome is also the use of the Internet, and direct-to-the-consumer sales, when the objectivity may be obscured by vested interests and unfounded opinions. Therefore it is essential that evidenced-based information is readily available to the patients or caregivers, including that about safety, long-term side-effects, adverse reaction rates and other data from high-quality clinical trials.

There is an increasing tendency for results from hardly relevant and reliable studies to be presented in support of placebos and potentially harmful agents marketed as evidence-based medications. Theoretical concepts are sometimes created, or existing ones misused for this purpose. Probably the best known example is hormesis: a concept of biphasic dose-response to different pharmacological and toxicological agents. According to this concept, a noxious agent at a small dose can exert a beneficial action, e.g. low dose stimulation and high dose inhibition. Hormesis has often been generalized without a plausible scientific basis, being used e.g. as a theoretic support for homeopathy. Some publications generalizing the hormesis phenomenon can be cited and used in support of homeopathy and placebos: in gerontology and other fields of medicine, also to endorse official registration of drugs and dietary supplements. Even known hormetic effects can be questionable because of the difficulties of differentiation between the low-level hormetic and placebo effects [1]. Moreover, due to an unfounded use of the hormesis concept, policy regulators such as the FDA or EPA may find themselves in a quandary about threshold levels for health risk.

Marketing of placebos in the guise of evidence-based medications has its social backgrounds: increasing costs of modern evidence-based treatments motivates to propagate less expensive substitutes. Several examples have been discussed previously: supplementation of glycosaminoglycans and their precursors in osteoarthritis [2], endobronchial surfactant supplementation for tuberculosis and other lung diseases without primary surfactant deficiency [3], sugar cane policosanols for atherosclerosis [4] and other [5]. Here is discussed the testing of serum atherogenicity in cell cultures used for registration and marketing of antiatherogenic drugs and dietary supplements.

Testing of Atherogenicity in Cell Cultures: Questionable Results and Arbitrary Interpretations

As in most biological research, the choice of animal or cell line model is of fundamental importance if extrapolations to the humans are intended. In this paper we draw attention to the limitations of the cell culture methods used for testing of serum atherogenicity and evaluation of antiatherogenic drugs. A cell culture is an artificial system in the sense that cultures developed on flat surfaces are hardly representative of the tissue milieu in vivo, reflecting, more or less correctly, only a small section in the complex interrelationships in a human body. Although 3-dimensional cultures in gel matrices or spheroidal clusters may be closer to the 'tissue' situation in terms of cell-to-cell signaling etc., they are still not a substitute for the in vivo conditions. Admittedly, the use of cellular systems in pharmacological studies is of great importance for the initial evaluation of drugs. However, their use to predict the response of the whole body to a given agent is limited. The determination of the concentration of a drug inhibiting a given physiological pathway in a cellular system bears little connection to the response of the body where apart from a direct cellular response to the drug there is interplay between different tissues and organs under the conditions of in vivo pharmacokinetics [6]. Moreover, as discussed below, the response of cellular systems to an agent affecting the transport across the plasma membrane can have opposite effects in the cell culture and the whole body. For these reasons, development of pharmacological treatments must be in the end based on in vivo animal studies followed by carefully designed clinical trials [6]. In any case, cell culture experiments need a scientifically correct interpretation, which is, as discussed below, not always the case.

An example is presented as follows viz. a large series of studies on serum atherogenicity, which became internationally known in 1986 after an article from the Cardiology Research Center in Moscow had

been published in The Lancet [7] followed by numerous publications and reports on meetings, continued until the present day [8-10] without references to the published criticism [11-13].

According to these reports, cultures of smooth muscle cells and macrophages were used for testing of serum atherogenicity, and the antiatherogenic effect of different substances was evaluated. For example, the following was reported.

1. Cell cultivation with diluted sera from patients with atherosclerosis caused a two- to five-fold elevation of intracellular cholesterol (Ch) [14].
2. LDL from atherosclerosis patients induced a two- to four-fold elevation of cholesteryl esters in the cultured cells [15,16].
3. Cultivation with sera or LDL from control subjects induced no lipid accumulation in the cells [14, 15].
4. Tested calcium antagonists reduced the Ch level in cultured cells and decreased incorporation of 3H-thymidine [17].
5. Tested beta-blockers caused a 1.5- to 2-fold increase in Ch content in cultured cells and stimulated their proliferation [17].
6. Garlic extract added to the culture medium significantly reduced the level of intracellular lipids in cultured smooth muscle cells and inhibited their proliferation. Ex vivo, blood serum taken from coronary atherosclerosis patients after an oral administration of garlic preparation significantly lowered Ch accumulation in the cultured cells [18].

All the above was interpreted as a direct antiatherogenic effect with inhibition of cell proliferation by certain pharmacological agents and natural substances. Apart from addition of tested substances to the culture medium, an ex vivo model was used: for example, an oral administration of propranolol rendered serum atherogenic i.e. capable of induction of intracellular Ch accumulation. At the same time, serum from patients receiving calcium antagonists acquired antiatherogenic properties, manifested by its ability to lower intracellular Ch and to inhibit proliferation of cultured cells; the same effect was observed also after direct admixture of the above-named drugs to the culture medium [17]. Remarkably, accumulation of lipids by cells was shown to be associated with an increase in their proliferation [17, 18]: e.g., in the cultures of smooth muscle cells taken from the areas of fatty infiltration and fatty streaks in the human aortic intima, the thymidine index exceeded the normal value 4.5- and 3-fold respectively [19]. It means that pharmacological agents influenced cell proliferation and intracellular lipid accumulation in the same direction [20]. However, in general pathology, fatty infiltration is considered to be a manifestation of cell damage or degeneration, which can hardly be accompanied by enhanced cell proliferation.

Apart from garlic, many natural substances have been shown by the methods described above to be effective against serum atherogenicity: leech salivary gland secretion, components of tea, black elder berries, calendula and violet flowers [21], grape seeds extract, fragmented grape stems, hop cones [22], etc. Squid liver fat and krill meat also produced “a marked reduction of blood serum atherogenicity” [23]. Extracts from 13 different mushrooms were shown to lower serum atherogenicity [24]. The method was applied for evaluation of sex hormones: estrogens and testosterone reduced serum atherogenicity and suppressed proliferation of cultured cells [25,26], whereas dihydrotestosterone had an opposite effect [25]. Relevance of the serum atherogenicity concept was confirmed quantitatively: a significant correlation was found between serum atherogenicity and the “increase of intima-media thickness of common carotid arteries” [27]. Furthermore, the “spontaneous upraise of serum atherogenicity during follow-up was contingent with progression of atherosclerosis ($P=0.008$)”; while the “complete removal of serum atherogenic potential in treated patients was contingent with atherosclerosis regression ($P=0.014$).” [27]. The authors claimed to have developed a “novel principle of direct antiatherosclerotic therapy based on inhibition of cholesterol deposition in arterial wall” [27]. As an explanation, circulating LDL-containing immune complexes were put forward. Ch content within circulating immune complexes was reported to correlate with the degree of coronary atherosclerosis. Accuracy of the coronary heart disease (CHD) prediction on the basis of Ch level within immune complexes was reported to be as high as 78 %; being

even higher for extra-coronary atherosclerosis [28]. Removal of IgG and IgM from serum was reported to lower its atherogenicity; while removal of circulating immune complexes eliminated it almost completely. It was concluded that immune complexes are one of the main sources of lipids, infiltrating the vessel wall leading to atherosclerosis [29]. Furthermore, a statistically significant correlation was found between serum concentration of anti-LDL antibodies and serum atherogenicity determined in the cell cultures [30]. The conclusion was that serum atherogenicity in CHD is caused predominantly by the Ch-containing immune complexes [29]. However, it is assumed that the possible atherogenic effect of immune complexes is mediated by damage to endothelium with a release of pro-inflammatory mediators [31]. Neither endothelium damage nor inflammatory phenomena were reproduced in the cell culture experiments. In other papers by the same authors, serum atherogenicity was explained by various LDL modifications: oxidation with aggregation [32], desialylation [33, 34], elevated negative electric charge [35], irreversible association [36], conformational modification of apo-B [37], and others. In contrast to the native LDL, modified LDL was reported to induce intracellular lipid accumulation in vitro [34]. However, the LDL modifications provide no plausible explanation for serum atherogenicity in cell cultures. For example, electronegative LDL were reported to release pro-inflammatory mediators from endothelium, in this way contributing to atherogenesis [38]. LDL with low sialic acid content were reported to bind more avidly to extracellular proteoglycans in the arterial wall [39]. Neither endothelium nor extracellular matrix was present in the cell cultures.

Discussion

Known action mechanisms of antiatherogenic or lipid-lowering drugs include regulation of Ch synthesis, of lipid metabolism in the liver, intestinal lipid absorption or of endothelial functions [40-43]. All these targets are absent in the cell cultures. Furthermore, there is considerable evidence in favor of the role of inflammation and autoimmunity in the atherogenesis [44]. Inflammatory phenomena, addressed by some antiatherogenic agents [45, 46], are also not reproduced by this model. Other systems and mechanisms participating in atherogenesis, such as coagulation and anticoagulation systems, platelets, hemodynamic factors, are absent in the cell cultures. Finally, studying serum atherogenicity, it should be considered that dyslipidemia in many CHD patients can be found only after meals: the so-called postprandial lipid abnormalities [47]. Diurnal fluctuations of serum lipid levels were not taken into account in the experiments with sera and cell cultures discussed above.

In vivo, the relationship between Ch uptake by cells and atherogenesis is inverse rather than direct. For example, in familial hypercholesterolemia, caused by abnormality of lipoprotein receptors, ineffective clearance of LDL-Ch from serum causes a Ch increase in blood and predisposition to atherosclerosis [48]. Downregulation of LDL-receptors contributes to insufficient clearance of lipoproteins from blood in nephrotic syndrome [49]. Another condition predisposing to atherosclerosis is caused by an apoE abnormality accompanied by lowered Ch uptake by cells [50]. By analogy with these examples, if a pharmacological agent reduces Ch uptake by cells in vitro, it must be expected to cause blood Ch elevation in vivo.

Conclusion

Considering the above, conclusions and recommendations for praxis, including the exact drug dosages [51], based on the cell culture studies discussed here, are unfounded and potentially misleading. Research quality and possible influence by the industry should be taken into account defining inclusion criteria for such studies into meta-analyses and systematic reviews. Scientifically inadequate methods can be used for the purpose of official registration of drugs and dietary supplements. This appears to be a general trend in today's science due to the insatiable multi-media appetite for medical breakthroughs and willingness of some researchers to produce corresponding materials. As a result, supposedly antiatherogenic and other medications with unproven effects are offered to the elderly patients misinformed not only by advertising but also

by some publications supposed to be scientific and professional. In this connection, intensive investigation before communication of information to the patients, doctors and wider community should always take place, whereas a conditio sine qua non is the researchers' integrity.

Acknowledgment

The paper was presented at the Healthy Aging Meeting, sponsored by the Dubbo Study of Australian Elderly on March 30, 2012 (Chairman, Prof. Leon Simons), a satellite meeting of The XVI International Symposium on Atherosclerosis (ISA2012) in Sydney, Australia. The author is sincerely grateful to the participants for a valuable discussion used in preparation of this article.

References

[1] Pickrell JA, Oehme FW. Examining the risks and benefits of replacing traditional dose-response with hormesis. *Hum Exp Toxicol* 2005; 24: 259-64.

[2] Jargin SV. Supplementation of glycosaminoglycans and their precursors in osteoarthritis versus diet modification. *Int J Rheum Dis* 2012; 15:e45-6.

[3] Jargin SV. Surfactant preparations for tuberculosis and other diseases beyond infancy: a letter from Russia. *Tuberculosis* (Edinb) 2012; 92:280-282.

[4] Jargin SV. Discussion of Evaluation of cholesterol-lowering and antioxidant properties of sugar cane policosanols in hamsters and humans. *Appl Physiol Nutr Metab* 2009;34:75.

[5] Jargin SV. Scientific misconduct or how to make the elderly pay for placebos. LAP Lambert Academic Publishing, Saarbrücken, 2013.

[6] Escobales N. Testing of anti-atherogenic drugs and food components on cell cultures: assessment of reliability. Author's reply. *P R Health Sci J*. 2010;29(1):87.

[7] Chazov EI, Tertov VV, Orekhov AN, Lyakishev AA, Perova NV, Kurdanov KA, Khashimov KA, Novikov ID, Smirnov VN. Atherogenicity of blood serum from patients with coronary heart disease. *Lancet* 1986;2:595-598.

[8] Sobenin IA, Chistiakov DA, Bobryshev YV, Orekhov AN. Blood atherogenicity as a target for anti-atherosclerotic therapy. *Curr Pharm Des* 2013;19 (33):5954-5962.

[9] Orekhov AN. Direct anti-atherosclerotic therapy; development of natural anti-atherosclerotic drugs preventing cellular cholesterol retention. *Curr Pharm Des* 2013;19(33):5909-5928.

[10] Orekhov AN, Sobenin IA, Korneev NV, Kirichenko TV, Myasoedova VA, Melnichenko AA, Balcells M, Edelman ER, Bobryshev YV. Anti-atherosclerotic therapy based on botanicals. *Recent Pat Cardiovasc Drug Discov* 2013;8(1):56-66.

[11] Jargin SV. Testing of anti-atherogenic drugs and food components on cell cultures: assessment of reliability. *P R Health Sci J*. 2010;29(1):86-7.

[12] Jargin SV. Testing of serum atherogenicity on cell cultures: assessment of reliability. *Gazz Med Ital* 2011;170:159-163.

[13] Jargin SV. Phytoestrogens and other botanicals: on the problems of evidence-based evaluation. *Recent Pat Cardiovasc Drug Discov* 2013;8(1):67-71.

[14] Orekhov AN, Tertov VV, Pokrovsky SN, Adamova IYu, Martsenyuk ON, Lyakishev AA, Smirnov VN. Blood serum atherogenicity associated with coronary atherosclerosis. Evidence for nonlipid factor providing atherogenicity of low-density lipoproteins and an approach to its elimination. *Circ Res* 1988;62:421-429.

[15] Tertov VV, Orekhov AN, Sobenin IA, Gabbasov ZA, Popov EG, Yaroslavov AA, Smirnov VN. Three types of naturally occurring modified lipoproteins induce intracellular lipid accumulation due to lipoprotein aggregation. *Circ Res* 1992;71:218-228.

[16] Tertov VV, Orekhov AN, Martsenyuk ON, Perova NV, Smirnov VN. Low-density lipoproteins isolated from the blood of patients with coronary heart disease induce the accumulation of lipids in human aortic cells. *Exp Mol Pathol* 1989;50:337-347.

[17] Orekhov AN, Baldenkov GN, Tertov VV, Ryong LH, Kozlov SG, Lyakishev AA, Tkachuk VA, Ruda MYa, Smirnov VN. Cardiovascular drugs and atherosclerosis: effects of calcium antagonists, beta-blockers, and nitrates on atherosclerotic characteristics of human aortic cells. *J Cardiovasc Pharmacol* 1988;12 Suppl 6:S66-68.

[18] Orekhov AN, Tertov VV, Sobenin IA, Pivovarova EM. Direct anti-atherosclerosis-related effects of garlic. *Ann Med* 1995;27:63-65.

[19] Orekhov AN, Kosykh VA, Repin VS, Smirnov VN. Cell proliferation in normal and atherosclerotic human aorta. II. Autoradiographic observation on deoxyribonucleic acid synthesis in primary cell culture. *Lab Invest* 1983; 48:749-754.

[20] Orekhov AN, Tertov VV, Kudryashov SA, Khashimov KhA, Smirnov VN. Primary culture of human aortic intima cells as a model for testing antiatherosclerotic drugs. Effects of cyclic AMP, prostaglandins, calcium antagonists, antioxidants, and lipid-lowering agents. *Atherosclerosis* 1986; 60:101-110.

[21] Gorchakova TV, Myasoedova VA, Sobenin IA, Orekhov AN. The reduction of serum atherogenicity by natural anti-inflammatory drugs. Abstracts of the XIV

International Symposium on Atherosclerosis, 18-22 June 2006, Rome. *Atherosclerosis Suppl* 2006; 7(3):230.

[22] Korennaya VV, Myasoedova VA, Nikitina NA, Sobenin IA, Orekhov AN. Bioflavonoid-rich botanicals reduce blood serum atherogenicity in perimenopausal women. Abstracts of the XIV International Symposium on Atherosclerosis, 18-22 June 2006, Rome. *Atherosclerosis Suppl* 2006;7(3):444.

[23] Shagaeva MM, Slavina LS, Balabolkin MI, Sobenin IA, Orekhov AN. Effect of squid liver fat and krill meat on blood serum atherogenicity in patients with type I diabetes mellitus. *Probl Endokrinol* (Mosk) 1993;39(4):21-23.

[24] Ryong LH, Tertov VV, Vasil'ev AV, Tutel'yan VA, Orekhov AN. Antiatherogenic and antiatherosclerotic effects of mushroom extracts revealed in human aortic intima cell culture. *Drug Dev Res* 1989;17:109-117.

[25] Kireev R.A., Kuvshinova E., Andrianova I.V., Sobenin I.A., Orekhov A.N. (2006). Effect of sex hormones on atherosclerotic indices in primary culture of female cells. Abstracts of the XIV International Symposium on Atherosclerosis, 18-22 June 2006, Rome. *Atherosclerosis Supplements*; 7(3): 250 (Abstract Tu-P8:296).

[26] Aksenov D., Orekhova V., Kireev R., Andrianova I., Smirnov A., Sobenin I., Panasenko O. (2008). Effect of sex hormones on intracellular cholesterol accumulation and LDL aggregation. Abstracts of the 77th Congress of the European Atherosclerosis Society, 26-29 April 2008, Istanbul. *Atherosclerosis Supplements*; 9(1): 20 (Abstract PO2-11).

[27] Orekhov AN, Sobenin IA. Serum atherogenicity predicts the progression of atherosclerosis. Abstracts of the XIV International Symposium on Atherosclerosis, 18-22 June 2006, Rome. *Atherosclerosis Suppl* 2006;7(3):209.

[28] Orekhov AN, Kalenich OS, Tertov VV, Perova NV, Novikov IyD, Lyakishev AA, Deev AD, Ruda MYa. Diagnostic value of immune cholesterol as a marker for atherosclerosis. J Cardiovasc Risk 1995;2:459-466.

[29] Tertov VV, Orekhov AN, Sayadyan KS, Serebrennikov SG, Kacharava AG, Lyakishev AA, Smirnov VN. Correlation between cholesterol content in circulating immune complexes and atherogenic properties of CHD patients' serum manifested in cell culture. *Atherosclerosis* 1990;81(3):183-189.

[30] Kacharava AG, Tertov VV, Orekhov AN. Autoantibodies against low-density lipoprotein and atherogenic potential of blood. *Ann Med* 1993;25(6):551-555.

[31] Virella G, Lopes-Virella MF. Atherogenesis and the humoral immune response to modified lipoproteins. *Atherosclerosis* 2008;200(2):239-246.

[32] Panasenko OM, Mel'nichenko AA, Aksenov DV, Tertov VV, Kaplun VV, Sobenin IA, Orekhov AN. Oxidation-induced aggregation of LDL increases their uptake by smooth muscle cells from human aorta. *Bull Exp Biol Med* 2007;143:200-203.

[33] Tertov VV, Kaplun VV, Sobenin IA, Boytsova EY, Bovin NV, Orekhov AN. Human plasma trans-sialidase causes atherogenic modification of low density lipoprotein. *Atherosclerosis* 2001;159:103-115.

[34] Tertov VV, Kaplun VV, Sobenin IA, Orekhov AN. Low-density lipoprotein modification occurring in human plasma possible mechanism of in vivo lipoprotein desialylation as a primary step of atherogenic modification. *Atherosclerosis* 1998;138:183-195.

[35] Tertov VV, Sobenin IA, Kaplun VV, Orekhov AN. Antioxidant content in low density lipoprotein and lipoprotein oxidation in vivo and in vitro. *Free Radic Res* 1998;29:165-173.

[36] Suprun IV, Mel'nichenko AA, Sobenin IA, Panasenko OM, Orekhov AN. Resistance of native and circulating modified low-density lipoproteins in human blood to association. *Bull Exp Biol* Med 2004;138:380-383.

[37] Suprun IV, Mel'nichenko AA, Yanushevskaya EV, Vlasik TN, Sobenin IA, Panasenko OM, Orekhov AN. Antigenic differences between apo-B in native and circulating modified low-density lipoproteins. *Bull Exp Biol Med* 2004;138:42-44.

[38] Sánchez-Quesada JL, Camacho M, Antón R, Benítez S, Vila L, Ordóñez-Llanos J. Electronegative LDL of FH subjects: chemical characterization and induction of chemokine release from human endothelial cells. *Atherosclerosis* 2003;166:261-270.

[39] Camejo G, López A, López F, Quiñones J. Interaction of low density lipoproteins with arterial proteoglycans. The role of charge and sialic acid content. *Atherosclerosis* 1985;55:93-105.

[40] Kraemer BF, Miller JW. Dyslipidemias. In: Carruthers SG, Hoffman BB, Melmon KL, Nierenberg DW, eds. *Melmon and Morrelli's clinical pharmacology: basic principles in therapeutics.* 4th Ed. New York, McGraw-Hill, 2000:552-580.

[41] Tomasoni L, Sitia S, Borghi C, Cicero AF, Ceconi C, Cecaro F, Morganti A, De Gennaro Colonna V, Guazzi M, Morricone L, Malavazos AE, Marino P, Cavallino C, Shoenfeld Y, Turiel M. Effects of treatment strategy on endothelial function. *Autoimmun Rev* 2010;9:840-844.

[42] Gebbers JO. Atherosclerosis, cholesterol, nutrition, and statins - a critical review. *GMS Ger Med Sci* 2007; 5:Doc04.

[43] Shah HD, ParikhI KH, Chag MC, Shah UG, Baxi HA, Chandarana AH, Naik AM, Shah JN, Shah KJ, Goyal RK. Beneficial effects of addition of fenofibrate to statin therapy in patients with acute coronary syndrome after percutaneous coronary interventions. *World Heart J* 2010;1(1):69-77.

[44] Jan M, Virtue AT, Pansuria M, Jingshan Liu, Xinyu Xiong, Pu Fang, Shu Meng, Hong Wang, Xiao-Feng Yang. The Role of Immunogenicity in Cardiovascular Disease. *World Heart J* 2011;3(1):1-29.

[45] Mundo-Sagardía JA, Figueroa Y, Altieri PI, Banchs HL, Escobales N, Crespo MJ. The atherosclerotic plaque. *P R Health Sci J* 2008;27:241-246.

[46] Biasucci LM, Biasillo G, Stefanelli A. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. *Clin Chem Lab Med* 2010;48:1685-1691.

[47] Slyper AH. A fresh look at the atherogenic remnant hypothesis. *Lancet* 1992;340:289-291.

[48] Marais AD. Familial hypercholesterolaemia. *Clin Biochem Rev* 2004;25:49-68.

[49] Anderson S, Tank JE, Brenner BM. Renal and systemic manifestations of glomerular diseases. In: Brenner MB, ed. *Brenner & Rector's The Kidney 6th Ed*. Philadelphia, W.B. Saunders Co., 1990:1885-1889.

[50] Cotran RS, Kumar V, Collins T. Robbins' *Pathologic Basis of Disease*. Philadelphia, W.B. Saunders Co., 1990:418-509.

[51] Orekhov AN, Pivovarova EM, Sobenin IA, Yakushkin VV, Tertov VV. Use of cell culture for optimisation of direct antiatherogenic therapy with verapamil. *Drugs* 1992;44 Suppl 1:105-10.