厚生労働科学研究費補助金

臨床研究基盤整備推進研究事業

各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討に関する研究

（若手医師・協力者活用に要する研究）

平成 16~18 年度総合研究報告書

主任研究者　　井口昭久

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目　次

Ⅰ. 総合研究報告

各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討に関する研究

井口　昭久

（資料）

1. The treadmill exercise-tolerance test is useful for the prediction and prevention of ischemic events in elderly diabetics
(Journal of Diabetics and Its Complications 19:2005:264-268)
Hayashi T, Nomura H, Esaki T, Hattori A, Kano-Hayashi H, Iguchi A

2. Risks of CHD Identified by Different Criteria ofn Metabolic Syndrome and Related Changes of Adipocytokines in Elderly Post Menopausal Women
(Journal of Diabetics and Its Complications:in press)
Ding Qun-Fang, Hayashi T, Osawa M, Hirai H, Iguchi A

3. Nitric oxide(NO)is a new clinical biomarker of survival in the elderly patients and its efficacy might be nearly equal to albumin
(Nitric Oxide 16:2007:157-163)
Osawa M, Hayashi T, Nomura H, Funami J, Miyazaki A, Ignarro LJ, Iguchi A

厚生労働科学研究費補助金（臨床研究基盤整備推進研究事業）総合研究報告書

各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討(若手医師・協力者活用に要する研究)

主任研究者　井口　昭久（名古屋大学大学院医学系研究科老年科学教授）

研究要旨　各種高脂血症治療薬の糖尿病性心血管病進展予防効果と作用機序を検討した。全体研究としては、主任研究者として、代謝内分泌学,循環器学,老年学,臨床薬理学専門医14名,12施設,40関連病院からなる研究班を結成した。2)17年3月末までに自立している糖尿病患者(4014名)を登録した。3)事務局として全登録患者を集計し、prospective cohort試験として,虚血性心疾患発症,死亡/同入院,CVD,ASO発症総死亡をエンドポイントに検討した。75.9%の脂質異常者を認めた。18年12月に登録後平均1.9年間の成績を回収し、解析をすすめた(追跡率初年度98.8、２年度92%)。心血管病発症率(虚血性心疾患IHD,脳血管障害CVD)は全糖尿病例では年2.2%と比較的高かった。 4)また、そのうち名古屋大学にて糖尿病患者215例、名古屋地区関連病院で糖尿病426例を登録し、上記研究に主体的に参加した。初年度イベント発症率は名古屋大学にてIHD2.8%, CVD1.4%、その他0.5%と高率であり、２年度イベント発症率はIHD2.4%, CVD1.5%、その他0.5%、登録者が高齢（平均74歳、ADLは自立）である事が関与していると推測された。関連病院では、各々0.2%、0.9%。0.7%で有った（平均年齢63歳）　現在, I)(本邦又は欧米の)血清脂質管理値達成によるイベント予防効果,II)高脂血症病態(メタボリック症候群,閉経等)による差異,III)新規高脂血症薬の安全性と多面的作用、IV)医療経済効果を検討している。糖尿病合併高脂血症薬の使用基準提示を目標とする。7)個別項目では糖尿病患者における認知症発症にTNF αとLDLcholesterol が関与している事、surrogate markerとして血中のNO代謝物濃度が有意である事を見いだした。

A. 採択された研究事業での研究概要

背景) 本邦においては糖尿病罹患者が増加しており,高脂血症合併例の増加及び心血管合併症のリスクとしての大きさが注目されている。加齢そのものによっても高脂血症患者の頻度は増大する。糖尿病性心血管病変は耐糖能異常の段階から進行し,長期罹患者が増加している。糖尿病患者の死因としては心血管合併症によるものが最も多く予防法確立が急務である。一方、糖尿病合併高脂血症の治療効果は血糖降下療法を凌駕する可能性も欧米の大規模臨床試験で報告され、日本動脈硬化学会は糖尿病罹患者は血清 LDL-Cholesterol の管理目標値を Ｂ３以上 120　mg/dl 以下としている。さらに米国では100mg/dl 以下と推奨している。さらに、スタチン製剤をはじめとする高脂血症薬には血管への直接作用がある可能性が報告されている。 複数の生活習慣病を合併する患者の増加に伴う治療方策が必要となっている。全体研究としては、主任研究者として、代謝内分泌学,循環器学,老年学,臨床薬理学専門医 14 名,12 施設,40 関連病院からなる研究班を結成した。2)17 年 3 月末までに自立している糖尿病患者(4014 名),を登録した。当該研究では名古屋大学を中心とし、一部関連病院での症例もあわせて登録し経過をフォローし、全体研究の中心となるように努力した。高齢者糖尿病患者の血清脂質管理の実態を、高脂血治療薬投与及び動脈硬化学会ガイドライン準拠率を中心に検討した。

全体研究はprospective cohort試験として,虚血性心疾患発症,死亡/同入院,CVD,ASO発症総死亡をエンドポイントに検討した。 また、そのうち名古屋大学にて糖尿病患者215例、名古屋地区関連病院で糖尿病426例を登録し、上記研究に主体的に参加した。

初年度イベント発症率は、名古屋大学にて高率であり、登録者が高齢である事が関与していると推測された。全体研究は18年12月に登録後平均1.9年間の成績を回収し、解析をすすめた(追跡率初年度98.8、２年度92%)。心血管病発症率(虚血性心疾患IHD,脳血管障害CVD)は全糖尿病例では年2.2%と比較的高かった。 また、そのうち名古屋大学では２年度イベント発症率も高かった。 糖尿病患者における認知症発症にTNF αとLDLcholesterol が関与している事、surrogate markerとして血中のNO代謝物濃度が有意である事を見いだした。

B. 採択された研究事業での研究実績

対象は一昨年度登録した、全国12ケ所,40関連病院の共同研究機関より,当初計画より多い**糖尿病罹患者**4014名である。内、名古屋大学にて糖尿病患者215例、名古屋地区関連病院で糖尿病患者426例を登録し、上記研究に主体的に参加した。原則として外来通院者等の自立した成人の２型糖尿病患者で、上記の対象には心筋梗塞,脳梗塞罹患者は含めていない。特に名古屋大学では付属病院老年科で自立した高齢者を多く含めた。全体のプロフィールは糖尿病群では平均年齢(64.5歳),男女比(1.12), HbA1C7.2%, TC 206.3,TG 144.1, HDL-C 55.5 mg/dlで脂質異常者は77.8%であった。一方、名古屋大学では平均年齢74.1歳(ADLは原則自立)、男女比0.78、HbA1C6.9%, TC209.1mg/dlであった。初年度イベント発症率は、名古屋大学にて脳血管障害1.4%、虚血性心疾患2.8%、その他0.5%と高率であり、登録者が高齢（平均74歳、ADLは自立）である事が関与していると推測された。関連病院では、各々0.2%、0.9%。0.7%で有った（平均年齢63歳）

当該年度より年齢階層別,性別, 薬剤別＜スタチン製剤(約84%),フィブラート製剤(9%)等＞、更に到達脂質濃度別(日本動脈硬化学会基準達成度, 総コレステロール値で32.2%)に各々分類しprospective cohort studyとして評価検討を行っている。虚血性心血管病(心, 脳血管障害,ASO)発症,入院等をend pointとし,一般所見,脂質等の冠危険因子治療経過を追った。75才以上の高齢者（登録時自立）は自立度の変化も評価する事とした。薬剤効果は現在解析中であるが、LDL濃度が低い場合(120mg/dl未満)及び高い場合(140mg/dl以上)に同一濃度範囲であってもスタチン使用者のIHD+CVD(2年間の解析からは特にIHD)罹患率が低い事が見いだされた。医療経済学的解析も施行し、概略的推計では現行のLDL濃度(平均120mg/dl)を90mg/dlに下げると, 虚血性心疾患発症率,10年後の罹患者総数とも約40%減少する可能性が示唆された。さらに脳血管障害も発症率を約24%,１０年後罹患者数を約25%減少させる可能性が示唆された。個別研究は, 血管内皮機能,TNF α,NO代謝物等のバイオマーカー,インスリン抵抗性,痴呆発症等との関係を検討し成果が出ている。安全管理モニター(名大鍋島, 浜医大中島両教授)の管理を頂いている。

(倫理面への配慮)

いずれの施設でも、研究対象者となる協力者に対してインフォームドコンセントを徹底し、協力者の利益が損なわれる事がないように十分に留意した。本研究は名古屋大学医学部附属病院をはじめ共同研究者が所属する施設の倫理委員会に申請,承認後に施行されている。被験者には同意を書面で頂き,いつでも取り消しが可能である事を明記し,認知機能障害のある方は対象外としている。プライバシーは匿名化を行い個人名が特定化されないよう細心の注意をはかっている。

C. 考察

本研究の意義は具体的な糖尿病、高脂血症の治療指針の策定にあるが、更に、長寿社会,日本で増加する生活習慣病自体の合併、心及び脳血管障害予防は、総合診療学、老年科学の領域でも重要と考え、代謝内分泌学、循環器学、老年学、臨床薬理学の専門家により,研究班を結成した。

具体的な成果及び今後の発展は全体研究では、１）糖尿病患者の重症度別評価に加え、高脂血症患者はメタボリック症候群罹患者,前期高齢者,閉経後女性（閉経後脂質上昇）等の層別の、目標脂質濃度、推

奨薬剤を設定できる可能性を探っている。当該研究で明らかになりつつ有るのは、糖尿病罹患者の血糖コントロールは高齢者ではむしろ良好に推移している（加齢による腎機能低下の影響か）点であり、血清脂質コントロールの意義がイベント数の現れる可能性がある。prospective cohort とい強力な手段をとり、全体の症例数を 4000 まで増やした事で、イベントに対する各種高脂血症薬の単独作用と、脂質低下作用におうところを直接，間接作用として解析できる可能性が示唆されている。一方，実態としては欧米はおろか本邦の学会ガイドラインでさえ４０%以下の準拠率である事が判明した。特に糖尿病合併高脂血症患者の心脳血管イベント発症率は部分集計では 2.1%強に上り，特に名古屋大学では 4.7%と高率で、昨年末報告された MEGA,JELIS の約 0.5%に比し,リスクの大きさ,逆に言えば制御する必要性が示唆される。個別報告にも有るようにストロングスタチンは単剤でも目標値達成の可能性があるが，部分集計では 50%前後に留まった。適応症例がかなり重症高脂血症患者に偏っている可能性も示唆される。2）脳血管障害は,脂溶性スタチンにのみ効果を認める可能性がある。３）第２年度は、医療経済学者、疫学統計学者を班員に加えたため上記にみとめられる解析法が選択された。年々市場規模が増大している高脂血症薬の効果的,効率的な投与方法を提言する。個別研究では高齢者の自立度及び QOL 改善に対する高脂血症薬治療の有効性の可能性を探りたい。バイオマーカーの分析により、高リスク群のスクリーニング及び治療効果の判定に応用したい。高脂血症薬の作用機序として、脂質低下作用に加え、NO 利用化による血管内皮機能改善を直接的抗動脈硬化作用の一つとして推測しており、広義の分子標的治療薬としての可能性やテロメラーゼ等、老化関連酵素への関与の可能性も探りたい。

D. 健康危険情報

現在のところは認めない。

E. その他実施した臨床研究・治験の概要及び実績

厚生労働科学研究　難治性疾患克服研究事業原発性高脂血症に関する調査研究：高齢者糖尿病合併複合型高脂血症の実態調査（212例）

F. 研究発表

（1） 論文発表

2004-2006 年の私の業績と
梅垣君の業績を入れて下さい。

G. 知的財産権の出願、登録状況

特になし

臨床研究実施チームの組織

（1）臨床研究実施チーム（a 組）

16 年 10 月 1 日- 18 年 3 月 3 1 日

	①若手医師及び臨床研究協力者に対する指導者	②若手医師	③臨床研究協力者
氏　　名	林　登志雄	大澤　雅子	平井　寿子
分担した研究項目	診療,同意取得、検査,群分け処方	診療,同意取得検査,データ解析	検査、データ解析

研究実施場所 （部局）	名古屋大学医学部 附属病院老年科	名古屋大学医学部 附属病院老年科	名古屋大学医学部 附属病院老年科

18年4月1日- 19年3月31日

	①若手医師及び臨床研究協力者に対する指導者	②若手医師	③臨床研究協力者
氏名	林　登志雄	鈴木　麻里	平井　寿子
分担する研究項目	診療,同意取得、検査,群分け処方	診療,同意取得 検査,データ解析	検査、データ解析
最終卒業学校・卒業年次・学位及び専攻科目	信州大学医学部医学科昭和59年卒・医学博士・老年科学	愛知医科大学医学部医学科平成12年卒・医学士・老年科学	岐阜大学農学部生命科学科修士 平成10年卒・農学修士・老年科学

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The treadmill exercise-tolerance test is useful for the prediction and prevention of ischemic coronary events in elderly diabetics

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Abstract

Background: Approximately 80% of cases of ischemic heart disease (IHD) occur in patients with nonstenotic coronary arteries, and few studies have systematically assessed exercise testing (TMT) as a predictor of risk in the elderly. **Methods:** TMT was carried out using a protocol for the independent and active elderly (n=176). After 4.1±0.5 years follow-up, logistic regression analysis was performed for each coronary risk factor such as diabetes mellitus (DM) and hypercholesterolemia (HC). According to the results, patients were divided into Gp HC, hypercholesterolemic patients; Gp DM, diabetics; Gp HC+DM, hypercholesterolemic diabetics; and Gp C, nonhyperlipidemic and nondiabetics. Sensitivity and specificity of TMT for IHD (significant stenosis or acute coronary syndrome) were analyzed. **Results:** Odds ratios for each risk factors are as follows: DM, 4.167; HC, 4.485; and DM+HC, 8.652. Notably, TMT was 17.59. Age was a significant risk, but hypertension was not. Positive ischemic signs in TMT were observed in 52.7%, 28.6%, 33.3%, and 16.3% in the Gp HC+DM, HC, DM, and C groups, respectively. Only three participants complained of chest pain during the TMT. Significant stenosis was observed in 75.0%, 71.4%, 69.2%, and 60.0% of coronary angiography (CAG)-receiving patients of Gp HC, DM, HC+DM, and C. During the observation term, acute coronary syndromes occurred in 4.7%, 3.3%, 5.5%, and 0% of patients in the Gp HC, DM, HC+DM, and C groups, respectively. The sensitivity of TMT for IHD was higher than 66.7% and specificity was higher than 94.1% in each group. **Conclusion:** An exercise tolerance test in the elderly, especially for diabetics and hypercholesterolemic patients, is useful for the diagnosis of IHD.

1. Introduction

Recent mega-trials have revealed that strict control of complicated coronary risk factors such as hyperlipidemia is important for the prevention of diabetic vascular lesions (Jonsson, Cook, & Pedersen, 1999). Exercise stress testing is an accepted means of estimating and diagnosing cardiovascular disease, as well as of predicting cardiovascular and all-cause mortality (Gianrossi, Detrano, Mulvihill, et al., 1989). However, approximately 80% of cases of ischemic heart disease (IHD) occur in patients with nonstenotic coronary arteries, and these cases cannot be predicted by an exercise-tolerance test (Bezerra, Higuchi, Libby, Ramires, et al., 2001). Furthermore, few studies have systematically assessed exercise testing as a predictor of risk in the elderly. Diabetic coronary lesions are known to have long segmental narrowing, and the incidence of IHD seems to be especially increased in patients who have had diabetes for more than 10 years (Al-Attar, Mahussain, & Sadanandan, 2002; Stein, Weintraub, Gebhart, et al., 1995). We have speculated that an exercise-tolerance test would be useful for the evaluation and prevention of IHD in elderly diabetics, if it could be carried out in a safe manner. We therefore modified the protocol of the exercise burden for the treadmill exercise-tolerance test (TMT) to make it more suitable for elderly patients.

The present study focused on the relationship between the frequency of cardiovascular ischemia, the exercise-tolerance test, and coronary risk factors in the elderly.

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2. Research design and method

2.1. Patient selection

Between April 1997 and March 2000, 342 patients were enrolled in this study. All patients were ambulatory and were either referred to our geriatric clinic (Nagoya University Hospital) or enrolled in our hospital to receive educational hospitalization for diabetes. Among them, 176 patients who were older than 65 years and who underwent an exercise-tolerance treadmill test were prospectively enrolled and followed for 4.1±0.5 years (Table 1). All patients gave their informed consent to participate in this study. None of the patients had experienced a myocardial infarction in the 3 months prior to enrollment, and they were independently active in daily life, as determined by their Lawton and Berthal scores (Collin, Wade, Davies, & Horne, 1988; Lawton & Brody, 1969).

2.2. Protocol and method

TMT was performed according to a protocol for the elderly, which we adapted from a protocol used for veterans in the United States (Prakash, Myers, & Froelicher, 2001). We changed the test so that each step lasted 2 min due to the age-related limitation of exercise tolerance (Hagberg, 1994; Tamesis et al., 1993; Table 2). The chronotropic response to exercise was assessed by estimating the proportion of the heart-rate reserve (220-age) used at peak exercise (Lauer, Francis, Okin, et al., 1999). Ischemic changes in the treadmill test were diagnosed using the Minnesota protocol; in brief, 1.0 mm or more ST segment elevation or depression in two or more leads was identified as positive. Exercise tolerance was estimated as METS, which was calculated from the participant's TMT results, body weight, age, and estimated Vo2 at rest. Plasma lipid and glucose levels were also measured. The diagnosis of hypercholesterolemia (HC) and diabetes followed the guidelines of the American Heart Association and Diabetes Association (Krauss, Eckel, Howard, et al., 2000; Resnick, Harris, Brock, et al., 2000). This study was approved by our institutional review board.

2.3. Follow-up data/definition of adverse outcome

All patients were followed until April 2002, with the mean follow-up period being 4.1±0.5 years after the treadmill test. The outcome was determined from patient interviews, hospital chart reviews, and telephone interviews. An adverse outcome was defined as the finding of significant stenosis in coronary angiography (CAG) with or without coronary intervention, such as percutaneous coronary angioplasty or ischemic cardiac events in the follow-up term. Cardiac events were defined as cardiac death, nonfatal MI, and resuscitated ventricular fibrillation or tachycardia after the TMT. Only the most severe outcome was considered an endpoint. Twenty-nine patients were excluded because of patient or physician refusal to follow-up (n=13), an inability to repeat the exercise treadmill test safely due to hearing loss (n=2), or geographic relocation (n=14). A total of 147 elderly individuals could be followed, and data on their histories of ischemic coronary disease, results of CAG, medication, and other parameters were recorded (Table 1). Based on the odds ratios evaluated as described below, patients older than 65 years were divided into four groups: Gp HC, hypercholesterolemic patients (n=42); Gp diabetes mellitus (DM), diabetic patients (n=30); Gp HC+DM, hypercholesterolemic and diabetic patients (n=36); and Gp C, nondiabetic and nonhyperlipidemic patients (n=39).

Table 1
Profile of patients

Patients number	147 (Male 71, Female 76)
Age (years)	71.7+0.4
Hypercholesterolemia	78 (Male 38, Female 40)
Diabetes mellitus (DM)	66 (Male 32, Female 34)
Hypertension	78 (Male 40, Female 38)
[Hypercholesterolemia+DM]	[36 (Male 17, Female 19)]

Table 2
Protocol of treadmill test for elderly

Stage	1	2	3	4	5	6	7	8	9
Period (min)	2	2	2	2	2	2	2	2	2
Speed (miles/h)	1	2	2	2	2	2	2.5	3.3	3.3
Gradient (%)	0	0	5	10	15	20	20	20	25
METs	2.5	3	5	6	8	9	10	11	13

2.4. Statistical analysis

Continuous data were expressed as the means±S.D. Categorical variables were analyzed by the chi-square test or Fisher's Exact Test. Continuous variables within groups were analyzed by repeated measures using analysis of variance (ANOVA). The Student's t test was used to identify significant differences in means. Stepwise multiple logistic regression analyses were used to identify the independent predictors of outcome, as well as the additive prognostic values of the clinical data and the exercise treadmill test. Fisher's Exact Test was used to calculate odds ratios or the probability of detecting any variables included in the logistic regression analysis in patients with adverse outcomes relative to patients with good outcomes.

3. Results

The odds ratios of each risk factor as determined by logistic regression analysis are shown in Table 2. Briefly, the odds ratios were as follows: DM, 4.167; HC, 4.485; and DM+HC, 8.652 (P<.01, respectively). That of age was significantly high (2.953; P<.05), whereas that of hypertension was not significant (2.151; P=.053). Notably, the odds ratio for positive ischemic signs as evaluated by TMT was 17.59.

Table 3
Odds ratio and 95% CI of each risk factor by logistic regression analysis

Hypercholesterolemia	4.485* (1.495–12.28)
DM	4.167* (1.477–10.81)
DM+Hypercholesterolemia	8.652* (2.543–13.68)
Hypertension	2.151 (0.845–9.26)
Age	2.953**(0.985–10.36)
Positive finding in TMTest	17.590*** (6.77–47.02)

* $P<.01$.
** $P<.05$.
*** $P<.001$.

We therefore divided the patients into four groups (Table 3): Gp HC, hypercholesterolemic patients (n=42; 72.0±0.5 years old; LDL-C, 150.7±10.4 mg/dl; exercise tolerance, 6.4±0.2 METs); Gp DM, diabetic patients (n=30; 72.3±0.9 years old; HbA1C, 7.6±0.5 g/dl; disease duration, 12.0±1.2 years; 6.0±0.5 METs); Gp HC+DM, hypercholesterolemic and diabetic patients (n=36; 71.4±0.8 years old; LDL-C, 149.5±11.5 mg/dl; HbA1C, 7.0±0.3 g/dl; disease duration for diabetes, 12.9±1.1 years; 6.4±0.3 METs); and Gp C, nondiabetic and nonhypercholesterolemic patients (n=39; 71.6±0.9 years old; 6.2±0.4 METs). The mean age and the frequency of other coronary risk factor complications, such as hypertension, smoking, and others, were not significantly different among the four groups. The TMT-positive ratios were 28.6%, 33.3%, 52.7%, and 16.3% in participants from the Gp HC, DM, HC+DM, and C groups, respectively (Fig. 1). Only three participants complained of chest pain during the TMT test (two in Gp HC+DM and one in Gp HC), and all of them became symptom-free within 5 min after exercise; all other positive patients were symptom-free. The ratios of patients receiving CAG per TMT-positive patient within 8 months after TMT were 66.7%, 63.6%, 68.4%, and 62.5% in the Gp HC, DM, HC+DM and C groups, respectively. CAG was not done for the following reasons: (1) patient refusal, lack of understanding of the CAG, and/or coronary intervention due to risk (n=11); (2) a high risk of coronary intervention for other general diseases such as chronic renal failure or cerebral infarction (n=6); and (3) physician refusal due to the risk of coronary intervention or CAG because of cognitive impairment, and others (n=6). In some patients who did not receive CAG but were suspected to have stenotic lesion by other examinations, medication such as anti-platelets and/or NO donors, such as isosorbide dinitrate, was prescribed. More than 75% stenosis was observed in 75.0%, 71.4%, 69.2%, and 60.0% of CAG-receiving patients of the Gp HC, DM, HC+DM, and C groups, respectively, and coronary intervention was performed in all of these cases (Fig. 1). During the 4.1±0.5 years of observation, ischemic coronary diseases such as angina pectoris or acute myocardial infarction occurred in 4.7% (8.3), 3.3% (10.0), 5.5% (5.3), and 0% of patients in the Gp HC, DM, HC+DM, and C groups, respectively (the percentage for TMT-positive patients). Older patients (older than 75 years of age) had more events (7.3% vs. 0%) than did the relatively younger patients (65–74 years; $P<.001$). Cardiac death was significantly more frequent in older

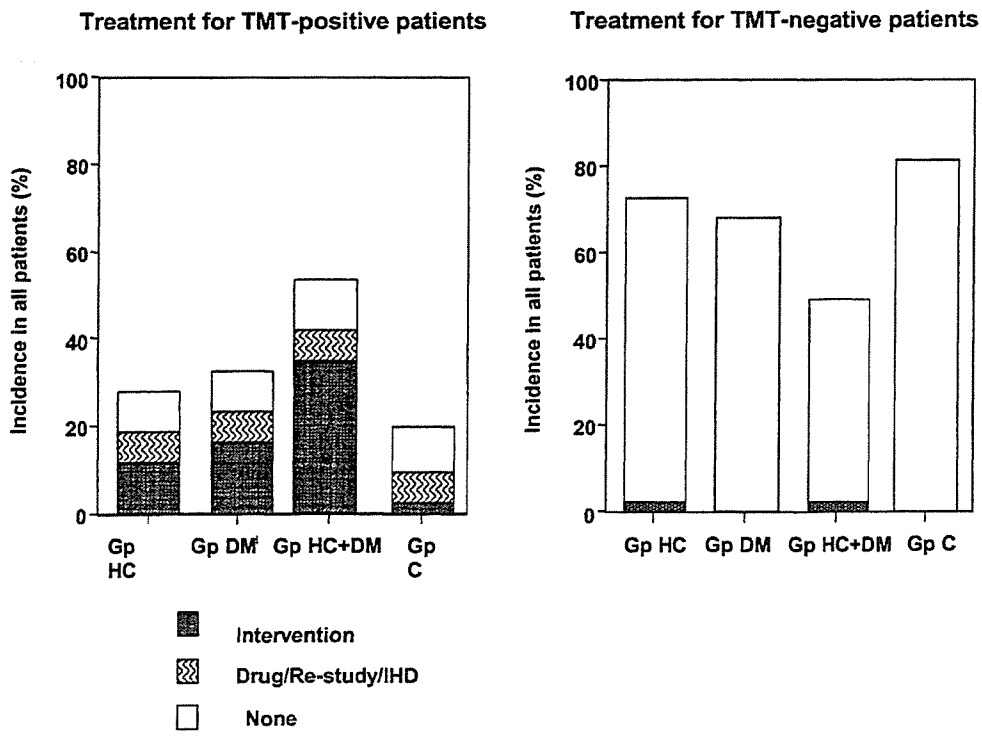

Fig. 1. Left: The frequency of TMT-positive findings and the corresponding treatments chosen for each disease group. Gp HC: hyper-cholesterolemic patients (n=42); Gp DM: diabetic patients (n=30); Gp HC+DM: hypercholesterolemic and diabetic patients (n=36); Gp C: nondiabetic and nonhypercholesterolemic patients (n=39). Right: The frequency of TMT-negative findings and the corresponding treatments chosen for each disease group.

Table 4
Patients profile who have coronary stenosis by CAG study, acute coronary syndrome, or drug treatment without CAG

	Percentage (%) of possible IHD				
	Total	In TMT-positive patients	In TMT-negative patients	Sensitivity of TMT for IHD	Specificity of TMT for IHD
Gp HC (42)	23.2	75.0	5.6	72.7≦	96.7
Gp DM (30)	26.7	72.7	0	66.7≦	100
Gp HC+DM (36)	41.7	73.7	5.9	68.8≦	94.1
Gp C (39)	10.3	66.7	0	66.7≦	100

Possible IHD means significant stenosis, ACS, and drug treatment during the observation term (4.1±0.5 years).
Sensitivity is calculated by (ACS and significant stenosis)/(TMT-positive patients−patients treated by drug without CAG).
Specificity is calculated by (no ACS or no significant stenosis)/(TMT-negative patients).

patients ($P<001$). Finally, significant stenosis observed by CAG, IHD, or medical intervention during follow-up term was observed in 75.0%, 72.7%, 73.7%, and 66.7% of TMT-positive patients in the Gp HC, DM, HC+DM, and C groups, respectively. Sensitivity and specificity were calculated as shown in Table 4, and they mean the reliability and usefulness of TMT for the diagnosis or speculation of IHD.

4. Discussion

The elderly population is increasing all over the world, and Japan is now the world's most aged society. Elderly individuals with IHD have higher rates of physical disability, as defined by a diminished ability to perform the activities of daily living, than do persons without IHD. Older age and clinical manifestations of angina pectoris or chronic heart failure are known to be associated with the highest rates of disability (Morey, Pieper, Crowley, Sullivan, & Puglisi, 2002). The odds ratio for age was also found to be significantly high in the present study (2.953; $P<.05$).

TMT using a protocol for the elderly was shown in the present study to be safe and possibly useful for maintaining independent activities of daily living in the elderly, as the positive ischemic signs evaluated by TMT showed an odds ratio of 17.59 despite the fact that 90% of patients testing positive were asymptomatic. The exercise tolerance (mean=6.1±0.5 METs) determined in the present study indicates that the elderly have the capacity to maintain the activities of daily living, including voidance and using the stairs. The optimal test duration is from 8 to 12 min, and the protocol workloads should be adjusted to permit this duration (Myers & Froelicher, 1993).

The odds ratios for each risk factor, as determined by logistic regression analysis, were the following: DM, 4.167; HC, 4.485; and DM+HC, 8.652 ($P<.01$, respectively). Hypertension, however, was not found to be significant (2.151; $P=.053$). Although the importance of diabetes as a coronary risk factor is well known, almost all patients with a positive TMT test were asymptomatic and showed a relatively high percentage of coronary stenosis. TMT is useful in screening for diabetic coronary macroangiopathy. The frequency of the TMT-positive ratio was found to be relatively high in the present study; we speculate that this finding was due to the fact that the study participants had suffered from diabetes for long periods and to our adoption of the standards of the AHA exercise-tolerance test (Gibbons, Balady, Basley, et al., 1997). We also examined 166 patients younger than 65 years as young control participants; these patients underwent TMT using a symptom-limited modified Bruce protocol and were followed for 4.0±0.8 years (data not shown). Their positive ratios were less than 15%, even in the patients with diabetes complicated with hyperlipidemia (data not shown). Despite a paucity of data on the predictive value of stress tests in older populations, current stress-testing guidelines extend the following recommendations to all adults aged 65 and older (Gibbons et al., 1997). The value of exercise training in patients older than 65 years is supported by a recent study involving 772 men with coronary heart disease, in which physical activity (walking, in particular) for a total of at least 4 hours per week was associated with a significant reduction in overall mortality. Thus, TMT should be useful in cardiac-rehabilitation programs for the elderly. Regarding the interpretation of these findings, a number of limitations should be mentioned. Goyara, Jacobsen, Pellikka, et al. (2000) found that exercise capacity, but not ST-segment changes, was predictive of mortality and cardiovascular events, but they did not distinguish patients who were older than 75 years of age from those who were younger. These findings do not agree with those of the present study, although we cannot identify the reason for this discrepancy. In our study, some patients did not undergo CAG due to patient or physician refusal, and others. Although the risk associated with CAG is small, some physicians cannot justify it fully to patients. This study was also confined to those patients who were referred for exercise testing and thus were able to walk on a treadmill. Despite these facts, our results demonstrated conclusively that TMT was useful for the prevention and management of ischemic coronary artery disease in elderly patients, especially in those with diabetes.

Especially, the high value of specificity of TMT means that TMT-negative finding means the less possibility of IHD and that TMT can be used as screening test of IHD for independent elderly.

References

Al-Attar, A. T., Mahussain, S. A., & Sadanandan, S. (2002). Cardiac tests in asymptomatic type 2 diabetics. *Medical Principles and Practice, 11*, 171–175.

Bezerra, H. G., Higuchi, M. L., Libby, P., Ramires, J. A., et al. (2001). Atheromas that cause fatal thrombosis is usually large and is frequently accompanied by vessel enlargement. *Cardiovascular Pathology, 10*, 189–196.

Collin, C., Wade, D. T., Davies, S., & Horne, V. (1988). The Barthel ADL Index: A reliability study. *International Disability Studies, 10*, 61–63.

Gianrossi, R., Detrano, R., Mulvihill, D., et al. (1989). Exercise-induced ST depression in the diagnosis of coronary artery disease. A meta-analysis. *Circulation, 80*, 87–98.

Gibbons, R. J., Balady, G. J., Basley, J. W., et al. (1997). ACC/AHA guidelines for exercise testing: Executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Exercise Testing). *Circulation, 96*, 345–354.

Goyara, T. Y., Jacobsen, S. J., Pellikka, P. A., et al. (2000). Prognostic value of treadmill exercise testing in elderly persons. *Annals of Internal Medicine, 132*, 862–870.

Hagberg, J. M. (1994). Exercise assessment of arthritic and elderly individuals. *Bailliere's Clinical Rheumatology, 8*, 29–52.

Jonsson, B., Cook, J. R., & Pedersen, T. R. (1999). The cost-effectiveness of lipid lowering in patients with diabetes: Results from the 4S trial. *Diabetologia, 42*, 1293–1301.

Krauss, R. M., Eckel, R. H., Howard, B., et al. (2000). AHA dietary guidelines: Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation, 102*, 2284–2299.

Lauer, M. S., Francis, G. S., Okin, P. M., et al. (1999). Impaired chronotropic response to exercise stress testing as a predictor of mortality. *Journal of the American Medical Association, 281*, 524–529.

Lawton, M. P., & Brody, E. M. (1969). Assessment of older people: Self-maintaining and instrumental activities of daily living. *Gerontologist, 9*, 179–186.

Morey, M. C., Pieper, C. F., Crowley, G. M., Sullivan, R. J., & Puglisi, C. M. (2002). Exercise adherence and 10-year mortality in chronically ill older adults. *Journal of the American Geriatrics Society, 50*, 1929–1933.

Myers, J., & Froelicher, V. F. (1993). Exercise testing. Procedures and implementation. *Cardiologia Clinica, 11*, 199–213

Prakash, M., Myers, J., & Froelicher, V. F. (2001). Diagnostic exercise tests on 4000 consecutive men. *American Heart Journal, 142*, 127–135.

Resnick, H. E., Harris, M. I., Brock, D. B., et al. (2000). American Diabetes Association diabetes diagnostic criteria, advancing age, and cardiovascular disease risk profiles: Results from the third national health and nutrition examination survey. *Diabetes Care, 23*, 176–180.

Stein, B., Weintraub, W. S., Gebhart, S. P., et al. (1995). Influence of diabetes mellitus on early and late outcomes after percutaneous transluminal coronary angioplasty. *Circulation, 91*, 979–989.

Tamesis, B., Stelken, A., Byers, S., Shaw, L., Younis, L., Miller, D. D., & Chaitman, B. R. (1993). Comparison of the asymptomatic cardiac ischemia pilot and modified asymptomatic cardiac ischemia pilot versus Bruce and Cornell exercise protocols. *American Journal of Cardiology, 1972*, 715–720.

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Risks of CHD Identified by Different Criteria of Metabolic Syndrome and Related Changes of Adipocytokines in Elderly Post Menopausal Women

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Abstract

The subject of this study was to assess the capacity of different criteria of metabolic syndrome (MetS) to identify risks of coronary heart diseases (CHD) and related changes of adipocytokines in post menopause women. A cross-sectional study was carried out in 225 community dwelled, elderly post menopause Chinese women without hormone replacement therapy (HRT). Blood pressure (BP), body mass index (BMI), serum lipid profiles, fasting glucose were analyzed and insulin sensitivity was estimated by homeostasis model assessment (HOMA-IR). Serum tumor necrosis factor alpha (TNFalpha), interleukin-6 (IL-6), and adiponectin were measured simultaneously. Prevalence of MetS identified by Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults(NCEP-ATPIII), International Diabetes Federation(IDF), Chinese Diabetes Society(CDS), and Japanese Society of Internal Medicine (JPN) were 27.31%, 37.34%, 23.29%, and 13.65%, respectively. No significant differences of baseline data were found among different MetS groups, except a significant higher waist circumference of JPN MetS group than other MetS groups. The prevalence of confirmed CHD in the four MetS groups were 26.2%, 18.6%, 26.9%, and 32%, respectively. Odds Ratios for CHD were 1.905(95% CI 1.273-2.851), 1.208 (95% CI 0.778-1.876), 1.997(95% CI 1.238-3.221), and 2.336(95% CI 1.119-4.876), respectively. JPN-MetS group had higher levels of TNF alpha and interleukin-6, whereas CDS-MetS group correlated better with lower adiponectin levels. Conclusion: IDF definition for MS is the most sensitive one to metabolic disorders, whereas JPN and CDS definitions correlate better with CHD and changes of adipocytokines.

Key words Metabolic syndrome; Cardiovascular diseases; Adipocytokines.

1. Introduction

Metabolic syndrome (MetS) is a cluster of metabolic disorders which has been convinced to be a strong predictor of diabetes mellitus (DM) and cardiovascular diseases (CVD) (Wilson et al, 2005). The first criteria was published by World Health Organization (WHO) in 1998 (Alberti, Zimmet for the World Health Organization, 1998), and then by the Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP ATPIII) in 2001(Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), and International Diabetes Federation (IDF) in 2005 (International Diabetes Federation, 2005). Comparing with NCEP ATPIII definition, IDF definition gives more weight to central obesity by taking it as the precondition of MetS diagnosis. Although the IDF definition has already considered of the ethnic characteristics of Asian by defining central obesity with lower levels of waist circumferences, it is still different from criteria published by Chinese Diabetes Society (CDS) (Chinese Diabetes Society, 2004) and Japanese Society of Internal Medicine (JPN) (Matuzawa et al., 2005) for their own populations.

Visceral adipose tissue plays an important role in the accumulation of metabolic disorders and chronic inflammations related to MetS by secreting adipocytokines, such as tumor necrosis factor alpha (TNFalpha), interleukin-6 (IL-6), prothrombin activator inhibitor-1 (PAI-1), and adiponectin. Adiponectin has been proved to play an unique role by its anti-atherogenic and insulin sensitizing properties (Lihn , Pedersen & Richelsen , 2005, Goldstein, Scalia, 2004). Lower adiponectin levels are associated with obesity and insulin resistance (Wayner et al., 2001) as well as higher risks of MI as an independent risk factor (Pischon et al., 2004, Nakayama et al.,2004).

Accumulated evidences have revealed closely correlations between aging in women and metabolic disorders as well as related cardiovascular diseases. Post menopause women (PMW) are more apt to visceral adipodropsy because of genetic as well as hormone milieu changes (Poehlman , Toth , &Gardner , 1995; Misso et al, 2005). Epidemiologic studies have revealed that prevalence of metabolic syndrome reaches to the highest level in women elder than 60 year-old in different ethnic group

(Reynolds , He , 2005; Vasilios et al, 2005; Gu et al, 2005). The same prone have also been showed in CVD morbidity and mortality (Kannel, McGee,1979). Thus, identification of risks of CHD in PMW by using proper criteria is virtually of crucial importance for earlier intervention.

The aim of this study was to compare the capacity of different criteria of MetS to predict risks of CHD and their associations with changes of adipocytokines in elderly post menopause women.

2. Methods

All participants were recruited in a cross-sectional investigation in local communities in Chengdu, China. All subjects were postmenopausal females (no menstruation for at least 1 year), aged ≥60 yr, without histories of hormone replacement therapy (HRT) or stopped HRT for at least 2 years. A group of doctors were responsible for collecting information from them at a direct interview in the outpatient clinics. Patients with cancers of reproductive system and in any acute stage of diseases were excluded. This study was approved by university ethic committee of Sichuan University.

Histories and related medications of CHD, type 2 diabetes mellitus, dyslipidemia, and hypertension were confirmed by a careful inquiring of the past history diagnosed.

Systolic and diastolic blood pressures (BP) were measured after resting for at least 20 minutes at a sitting position with standard up-standing mercury sphygmomanometer. Body weight, body height, waist circumferences (WC) and hip circumferences were carefully measured with the participants in light clothes and without shoes.

After an overnight fasting for 8-12 hours, blood samples were collected from each individual via venipuncture. Serums were separated by centrifuge and then stored in -20°C until analysis. Serum fasting glucose (FBG), total triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC) were measured by standardized hospital laboratory procedure.

Insulin sensitivity was estimated by calculating homeostasis model assessment (HOMA-IR) score (Matthews DR, 1985) with the following formula: fasting serum insulin × fasting serum glucose /22.5.

Serum adipocytokines (TNF alpha, IL-6, adiponectin) were measured by Quantifile ELISA kits (R&D systems, Minneapolis, MN) according to the manual.

Metabolic Syndrome was defined by following criteria:

NCEP- ATPIII definition:

At least 3 of the following abnormalities:

FBG≥110 mg/dL or taking medication for diabetes,

Abdominal obesity: WC >102cm in men or >88 cm in women

TG≥150 mg/dL, or HDL-C <40 mg/dL in men, <50 mg/dL in women,

BP≥130 or≥85 mm Hg or previous diagnosed hypertension.

IDF definition:

Central obesity (for Chinese women, waist circumferences ≥80 cm) plus any two of the following

TG ≥150 mg/dL (1.7 mmol/L) or HDL-c<40 mg/dL (1.03 mmol/L) in men<50 mg/dL (1.29 mmol/L) in women or specific treatment for this lipid abnormality;

BP ≥130 mmHg / ≥85 mmHg or treatment of previously diagnosed hypertension;

FBG≥100 mg/dL (5.6 mmol/L) or previously diagnosed diabetes.

CDS definition:

At least 3 of following abnormalities

(1) Body mass index (BMI) ≥25 kg/m2;

(2) BP ≥140/90 mmHg or under antihypertensive medication;

(3) TG≥1.7 mmol/L or HDL-C <0.91 mmol/L in males and <1.0 mmol/L in females;

(4) FBG level ≥6.1 mmol/L or under antidiabetic medication.

JPN definition:

Accumulated visceral adipose tissue (waist circumference≥90cm for women and ≥85 for men)

plus at least 2 of the following metabolic abnormalities:

(1) TG≥150mg/dl and or HDL-C≤40mg/dl;

(2) Systolic BP ≥130 and or diastolic BP≥85mmHg;

(3) FBG≥110mg/dl.

Under medications for the metabolic abnormalities mentioned above should also be included.

3. Statistical analysis

All data were expressed as mean±SD. Statistical analyses were performed by using Statistic Package for Social Science (SPSS, for windows, version 12, SPSS Inc, Chicago, Ill). TNA alpha, IL-6, and adiponectin data were logarithmized when analysis. Differences among groups were analyzed with one way ANOVA and post hoc analysis. Pearson's correlation coefficients were applied for relationships between parameters. The level of significance was set as $P<0.05$.

4. Results

The baseline data of participants were listed in table1. The mean age was 66.77±5.09 yrs, and mean years after menopause was 18.22±7.41 yrs. Compared with data reported, the mean levels of BMI, WC, systolic BP, diastolic BP, and TG were higher than average levels of women in south China (GU D F et al, 2005). The prevalence of MetS diagnosed by IDF, NCEP ATPIII, CDS and JPN criteria were 37.34%, 27.31%, 23.29%, and 13.65%, respectively. IDF-MetS had the highest prevalence, whereas JPN-MetS had the lowest one. Each MetS group had significant higher levels of parameters related to the components of MetS than the non-MetS group defined by the same definition, except a lower HDLC level ($p<0.05$) (data not shown here). There were no significant differences of parameters related to metabolic disorders among different MetS groups, except a higher WC in JPN-MetS group (As showed in table2). CDS MetS group had the highest levels of BMI, glucose, TG, and SBP; JPN MetS group had the highest levels of insulin, HOMA-IR, LDLC, waist and hip circumferences, and lowest levels of HDLC; whereas IDF MetS group had the lowest levels of insulin, HOMA-IR, TG,TC,BMI, and SBP, comparatively. No significant differences were revealed among non-MetS groups defined by different criteria (data not shown here).

To evaluate correlations of different criteria of MetS with CHD and DM, prevalence of diagnosed CHD and DM related to different MetS groups were analyzed (As showed in table 3A). And odds ratios for DM and CHD were calculated (results showed in table 3B). JNP-MetS group and CDS-MetS group had comparatively higher prevalence and ORs for CHD than other two groups. More than half of the CDS and NCEP-ATPIII defined individuals had established diagnosis of DM.

Although no significant changes of adipocytokines were found among different MetS groups, CDS MetS group tended to have the lowest levels of adiponectin, whereas JPN MetS group tended to have the highest levels of TNF alpha and IL-6. Prevalence of MetS defined by different criteria in the highest and lowest quartile of adiponectin were different (data shown in table 4).

5. Discussion

Our investigation revealed differences of prevalence of MetS defined by different criteria. The reasons should be the underlying principles and cutoff points of different definitions.

NCEP ATPIII definition for metabolic syndrome is the most widely used one, because of its convenience for clinical utilization. Identified by NCEP ATPIII criteria, prevalence of MetS in our study was 26.2%. It is in consistency with other large scale investigations (Gu DF et al, 2005). This group appeared to have higher TG, lower WC and TC than their Caucasians counterparts (Tankó, L. B. et al, 2005). A generally lower level of adiponectin was also noticed. We consider these as characteristics of MetS in Chinese PMW.

Comparing with NCEP ATPIII, IDF definition of MetS gives more weight to central obesity and lowers the cutoff point of waist circumference to 80cm (for Chinese women) and fasting glucose level to 5.6mmol/L, according to the suggestions of American Diabetes Society. It defined more MetS, to the level of 37.65%, and thus seemed to be more sensitive. This is in consistency with reports from other ethnic groups (Athyros VG, et al, 2005; Earl S.F, 2005). Most of those parameters related to metabolic disorders in MetS were revealed lower in this group than in other groups, except a higher HDLC.

CDS definition of MetS seems to be a modification and combination of both WHO and NECP-ATPIII definition by choosing a comparatively higher cutoff point for hypertension and selecting BMI as a parameter for central obesity. Prevalence of MetS defined by CDS was much lower than by IDF, but similar to that defined by NCEP ATPIII. Given that the similarities between NCEP ATPIII and CDS definitions, it may mean that either BMI>25 or WC>88 identify the same in terms of visceral adipodropsy.

Like IDF definition, the JPN definition also takes central obesity as a precondition of diagnosis, with higher cutoff points of WC, which is based on the visceral fat area exceeding 100cm2 in Japanese women (Matuzawa et al., 2005). Considering of ethnic identity and similarities in lifestyle, we applied JPN definition designed for Japanese women to our studied Chinese women. To our surprise, a sharply decrease of diagnosed MetS was revealed. Since the average WC of women in south China is only 75.6 cm as reported (Gu D F et al 2005), the cutoff points of 90cm of WC ruled out many cases whom had already had more than one metabolic disorders, and were certified to other criteria of MetS.

The dramatic change of prevalence of MetS diagnosed by different criteria in the same population give rises to an important

question: who should be treated from now? Since the final aim to define MetS is to identify individuals at high risks of CVD to whom earlier interventions are necessary, assessment of correlations of each definition with CVD and DM may help answer the previous question.

As reported in our results, NECP ATPIII definition seemed to correlate the best with DM, whereas JPN-MetS group seemed to correlated better with CHD. In MetS groups with higher CHD prevalence, like JPN-MetS group and CDS-MetS group, there were trends to have older ages, longer years after menopause, higher levels of TG, BMI, and systolic BP, although no statistical significances were revealed. We also noticed that although JPN-MetS group had the highest CHD prevalence, the prevalence of DM was the lowest and so were the levels of glucose. It also had the highest levels of waist circumferences and HOMA-IR index among the four groups. These give us such an impression that not levels of glucose but the underline basis of central obesity plays more important roles.

Adipocytokines may be the key factors link visceral adipose accumulation and atherosclerotic diseases, by playing harmful (eg.TNFalpha, IL-6) or protective (eg. adiponectin) roles. In our study, adiponectin levels were revealed to be significant higher in MetS groups than in normal groups. No significant differences were revealed between PMW with MetS and PMW with metabolic disorders but did not reach to the criteria of MetS. JPN-MetS group had the highest levels of both TNFalpha and IL-6. But we failed to reveal related lowest levels of adiponectin in this group. CDS-MetS group had the lowest levels of adiponectin. Multi-variants linear regression analysis revealed a significantly positive correspondence between TG and adiponectin, and a significantly negative correlation between HDLC and adiponectin (data not showed). TC and LDLC were revealed to significantly directly related to TNF alpha and IL-6 (data not showed). No significant correlations between adipocytokines and WC or BMI were figured out in our study. This may reflex the complication of this multiple metabolic disorder. Quartile analysis of adiponectin revealed, among all those PMW within the lowest quartile level of adiponectin, prevalence of MetS defined by CDS was the highest; while in the highest quartile, prevalence of MetS defined by JPN was the lowest (as showed in Table 4).

In San Antonio Heart Study, the differences between WHO and NCEP ATPIII criteria in predicting all-courses cardiovascular mortality were revealed (Hunt et al., 2004). Differences among different criteria in relation to confirmed CHD were revealed in our study. Our results may be limited by a comparatively small scale and the limitations of a cross-sectional study. Since this is just a primary report of an on-going multi-center, prospective study, we are looking forward to further results.

In conclusion: IDF definition for MetS is the most sensitive one to metabolic disorders, whereas JPN and CDS definitions correlate better with CHD and changes of adipocytokines. The supplementation of different dcriteria with adipocytokines may give more meanings to the definition of metabolic syndrome.

Reference

1. Alwan A, King H (ed): Report of a World Health Organization consultation. Definition, diagnosis and classification of diabetes mellitus. Part 1: diagnosis and classification of diabetes mellitus. World Health Organization, Department of Noncommunicable Disease Surveillance .Geneva, 1-59, 1999

1.Athyros VG, Ganotakis ES, Elisaf M, Mikhailidis DP: The prevalence of the metabolic syndrome using the National Cholesterol Educational Program and International Diabetes Federation definitions. Curr Med Res Opin. 21(8):1157-9, 2005

1.Expert Panel on Metabolic Syndrome of Chinese Diabetes Society. Recommendations on Metabolic Syndrome of Chinese Diabetes Society (Chinese). Chin J Diab 12:156–61. 2004

1.Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 285:2486–2497, 2001

1.Ford ES: Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes care 28: 2745-2749, 2005

1.Goldstein BJ, Scalia R: Adiponectin: A novel adipokine linking adipocytes and vascular function J Clin Endocrinol Metab.89(6):2563-8, 2004

1.Gu DF, Reynolds K, Wu XG, Chen J, Duan XF, Reynolds RF, Whelton PK, He J, for the InterASIA Collaborative Group: Prevalence of the metabolic syndrome and overweight among adults in China. The lancet 365: 1398-405, 2005

1.Hunt KL, Resendez RG, Williams K, Haffner SM, Stern MP: National Cholesterol Education Program Versus World Health

Organization Metabolic Syndrome in Relation to All-Cause and Cardiovascular Mortality in the San Antonio Heart Study Circulation 110: 1251-1257, 2004

1.Hunt KL, Resendez RG, Williams K, Haffner SM, Stern MP: National Cholesterol Education Program Versus World Health Organization Metabolic Syndrome in Relation to All-Cause and Cardiovascular Mortality in the San Antonio Heart Study Circulation 110: 1251-1257, 2004

1.International Diabetes Federation: The IDF consensus worldwide definition of the metabolic syndrome. Available from http:// www.idf.org/webdata/docs/Metabolicsyndrome-definition.pdf. Accessed 2 september 2005

1.Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study.JAMA 241:2035–2038, 1979

1.Lihn SA, Pedersen SB and Richelsen B: Adiponectin: action, regulation and association to insulin sensitivity. Obesity reviews 6: 13-21, 2005

1.Matsuzawa Y: Metabolic Syndrome-definition and diagnostic criteria in Japan. J Jpn Soc Int Med 94: 188-203, 2005

1.Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 7: 412–419, 1985

1. Misso ML, Jang C, Adams J, Tran J, Murata Y, Bell R, Boon WC, Simpson ER, Davis SR : Differential expression of factors involved in fat metabolism with age and the menopause transition Maturitas 51:299–306, 2005

1. Nakayama Y, Shimada K, Fukada Y, Ehara S, Hirose S, Kataoka T, Kamimori K, Shimodozono S, Kobayashi Y, Yoshiyama M, Takeuchi K, Yoshikawa J:Implications of plasma concentrations of adiponectin in patients with coronary artery diseases. Heart 90: 528-533, 2004

1.Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB: Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 291:1730–1737, 2004

1.Poehlman ET, Toth MJ, Gardner AW: Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann Intern Med123:673–5, 1995

1. Reynolds K, He J : Epidemiology of the metabolic syndrome Am J Med Sci 330(6): 273-279, 2005

1.Tankó, LB, Bagger YZ, Qin G, Alexandersen P, Larsen PJ, Christiansen C: Enlarged Waist Combined With Elevated Triglycerides Is a Strong Predictor of Accelerated Atherogenesis and Related Cardiovascular Mortality in Postmenopausal Women Circulation 111:1183-1890, 2005

1.Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86(5):1930-5, 2001

1.Wilson PWF, D'Agostino RB, Parise H, Sullivan L, Meigs JB: Metabolic Syndrome as a Precursor of Cardiovascular Disease and Type 2 Diabetes Mellitus. Circulation 112: 3066-3072, 2005

Table 1 Baseline data of PMW

Age(years)	66.77±5.09	BMI(kg/m2)	23.24±3.13
Age at menopause(years)	47.97±5.79	WC (cm)	80.07±14.94
years after menopause(years)	18.22±7.41	hip circumference(cm)	94.05±14.92
Glucose(mmol/L)	5.48±1.81	systolic Bp(mmHg)	132.16±18.44
insulin(uU/ml)	8.85±16.61	diastolic Bp(mmHg)	78.61±10.29
TG(mmol/L)	2.00±1.29	HOMA-IR	1.64±1.49
TC(mmol/L)	5.11±1.97	TNFalpha(pg/ml)	2.08±1.30
HDLC(mmol/L)	1.59±0.40	IL-6(pg/ml)	3.09±5.03

LDLC(mmol/L)	3.09±0.81	Adiponectin(ng/ml)	5731.25±3201.76

MetS, metabolic syndrome; TC, total cholesterol; TG, total triglyceride; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; BMI, body mass index; WC, waist circumference; BP, blood pressure; HOMA-IR, homeostasis model assessment for insulin resistance; TNF alpha, tumor necrosis factor alpha; IL-6, interleukin 6; PMW, post menopause women.

Table 2. Comparison of parameters among different MetS groups

	NCEPATPIII MetS	IDF MetS	CDS MetS	JAP MetS
age(years)	67.81±4.75	67.68±4.57	68.05±4.64	68.09±5.02
age at menopause(years)	46.91±7.70	46.84±6.7	47.19±7.03	45.53±9.65
years after menopause(years)	19.93±7.52	19.81±7.21	19.89±6.39	20.50±8.55
glucose(mmol/L)	6.34±1.98	6.17±2.12	6.50±2.01	6.04±1.89
Insulin(uU/mL)	13.66±28.71	12.59±25.33	16.00±31.46	16.7±39.29
HOMA-IR	3.97±9.28	3.49±8.08	4.62±10.02	4.99±12.77
TG(mmol/L)	2.75±1.56	2.55±1.58	2.84±1.82	2.70±1.51
TC(mmol/L)	5.05±1.09	5.07±0.87	5.08±1.12	5.02±1.10
HDLC(mmol/L)	1.428±0.33	1.50±.36	1.45±.322	1.39±0.36
LDLC(mmol/L)	2.99±0.87	2.98±0.83	2.97±0.92	3.00±0.92
BMI(kg/m2)	24.06±3.72	23.91±3.50	25.63±3.35	24.47±4.66
Waist circ(cm)	89.41±8.33	87.99±6.98	85.87±11.91	95.26±5.5*
hip circu(cm)	102.38	100.62±6.86	99.13±12.31	107.13±6.6
systoli Bp(mmHg)	138.04±18.69	135.85±16.9	142.04±18.77	137.1±18.5
diastol Bp(mmHg)	79.85±11.21	79.58±10.17	81.46±10.96	79.58±10.9
TNFalpha(pg/ml)	1.89±1.16	1.90±0.96	1.81±0.97	2.01±1.23
IL-6(pg/ml)	2.97±4.17	2.86±3.91	2.91±3.72	3.12±4.07
Adiponectin (ng/ml)	5840.05±3238.94	6274.77±3463.60	4887.74±2661.62	5899.13 ± 3953.67

* $p<0.05$

MetS, metabolic syndrome; TC, total cholesterol; TG, total triglyceride; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; BMI, body mass index; WC, waist circumference; BP, blood pressure; HOMA-IR, homeostasis model assessment for insulin resistance; TNF alpha, tumor necrosis factor alpha; IL-6, interleukin 6; PMW, post menopause women; NCEP-ATPIII,Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation; CDS, Chinese Diabetes Society; JPN, Japanese Society of Internal Medicine.

Table 3A Prevalence of CHD and DM in different MetS group

	Prevalence of CVD		Prevalence of DM	
	diagnosed	family history	diagnosed	family history
IDF-MS	18.60%	10.52%	39.61%	9.21%
NCEP-MetS	26.20%	4.68%	55.70%	6.25%

CDS-MS	26.90%	5.45%	50%	9.09%
JPN-MS	32%	7.40%	36%	7.40%

Table 3B ORs of CHD and DM in different MetS group

	Odds Ratio of CHD	95% CI	Odds Ratio of DM	95% CI
IDF-MetS	1.208	0.778-1.876	2.32	1.702-3.162
NCEP ATPIII-MetS	1.905	1.273-2.851	4.048	2.802-5.848
CDS-MetS	1.997	1.238-3.221	3.041	1.963-4.709
JPN-MetS	2.336	1.119-4.876	2.184	1.058-4.507

NCEP-ATPIII, Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation; CDS, Chinese Diabetes Society; JPN, Japanese Society of Internal Medicine; MetS, metabolic syndrome; CHD, coronary heart disease; DM, diabetes mellitus.

Table 4 Prevalence of MetS in lower and higher quartile of adiponectin

	adiponectine (pg/ml)	Prevalence of MetS according to different criteria(%)			
		IDF	NCEP ATPIII	CDS	JPN
lower quartile	301.07-3309.67	27.3	24.14	32.25	19.23
upper quartile	4751.31-7730.66	32.3	34.78	10.81	10

NCEP-ATPIII, Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation; CDS, Chinese Diabetes Society; JPN, Japanese Society of Internal Medicine; MetS, metabolic syndrome.

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Nitric oxide (NO) is a new clinical biomarker of survival in the elderly patients and its efficacy might be nearly equal to albumin

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Abstract

Background: For elderly patients, the consideration of prognostic factors is very important, but there have been few reports about the potential use of vasoactive substances as prognostic markers in the elderly.

Objective: We assessed endocrinological substances, such as plasma NO_x (metabolites of NO), as the prognostic marker in elderly. We compared their efficacy with that of such well-known markers as albumin and pro-inflammatory cytokines such as IL-6.

Methods: The patients were recruited consequently from the clinics of Nagoya University Hospital or related home care services facilities. One hundred and twenty seven elderly aged 65 and older were registered. Biochemical analyses such as albumin, total cholesterol, BNP, and NO_x were measured upon enrollment. The main outcome was the survival rate.

Results: Forty-six patients died during the follow-up period. Mann–Whitney's *U*-test showed that the levels of age, hemoglobin, total protein, serum albumin, serum creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, high sensitive CRP, NO_x, IL-6, and TNF-α were significantly different between the living and deceased subjects. Among the dependent variables in the logistic regression analyses, only albumin and NO_x were significantly different. In the Kaplan–Meier analyses of mortality, the prognosis of patients in 3rd and 4th quartile of NO_x was significantly worse than that in 1st or 2nd quartile.

Conclusion: NO_x has potential both as a vascular marker and as a marker for predicting survival in elderly. In the latter role, it may be as effective as albumin.

Keywords: Nitric oxide; cGMP; Albumin; Biomarker; Elderly; Prognostic marker; Vascular functional marker

Many nations, including Japan, are experiencing rapid growth in their elderly populations. The main causes of death in Japanese elderly are heart disease, cerebro-vascular disease, and cancer. Several biochemical markers, such as albumin and cholesterol, have been identified as having prognostic value for mortality and hospitalization [1–3]. Recent studies also have indicated the potential role of the immune system in the pathophysiology of congestive heart failure (CHF) and malignancy [4,5]. Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) also have been reported to be significant prognostic predictors in patients with CHF or malignancy) [6–8]. TNF-α induces adhesion molecule expression such as ICAM-1 on endothelial cells, which promotes the progression of atherosclerosis [9]. In other words, in older populations, peripheral blood markers of nutrition or inflammation (albumin, cholesterol, IL-6, and TNF-α) have been individually shown to be increased risk for mortality [2,10,11].

In elderly people, the rate of CHF is important for predicting mortality and hospitalization rates. Brain natriuretic peptide (BNP) is a good marker of CHF, because the plasma BNP concentration is elevated according to the severity of CHF [12–15]. Binding of BNP to its receptors

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initiates natriuretic and vasorelaxant activities through an elevation in intracellular cyclic guanosine monophosphate (cGMP) [16,17]. Nitric oxide (NO) is also an important vasoactive substance, because it exerts anti-atherogenic effects by inhibiting the migration or proliferation of monocytes or smooth muscle cells and vasodilation mainly by cGMP dependent mechanism [18]. We reported that NO regulates cGMP in patients with renal insufficiency [19]. NO may be a useful prognostic marker for patients suffering from atherosclerotic diseases such as cerebral strokes or myocardial infarction, although as yet there have been no reports investigating the use of NO in this capacity. The source of NO is not only endothelial cells (endothelial NO synthase; eNOS) but also macrophages or T cells (inducible NO synthase; iNOS) and some neuronal cells (neuronal NO synthase; nNOS). The plasma level of NO_x (nitrite plus nitrate, metabolites of NO) may reflect the status of eNOS and, to some extent, the status of iNOS. Because iNOS is activated in patients with inflammations such as sepsis, advanced stages of malignancy, or progressed atherosclerotic lesions, the NO_x level may have potential as a marker of malignancy as well as atherosclerotic diseases [20,21].

For elderly patients, the consideration of prognostic factors is very important, but there have been few reports about the potential use of vasoactive substances. Therefore, in this study, we evaluated whether measurements of plasma levels of vasoactive factors such as NO_x, cytokines such as IL-6, and well-known markers such as albumin were useful as prognostic factors in the elderly.

Methods

Study sample

One hundred and twenty seven elderly subjects (48 males and 79 females; mean age, 81.3 ± 7.5 years; range, 65–101 years) were enrolled on August on 2002. The study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine and written informed consent was obtained from all patients. Patients were selected consecutively among our geriatric clinics and related home care services. In brief, 91 participants were presented at Department of Geriatrics, Nagoya University Hospital and the related hospital as outpatients (31 from their homes, 31 from geriatric nursing care units, and 29 from other facilities such as private homes for the aged) and 36 were in home care services facility. At the baseline examination, participants underwent a review of their medical history, a physical examination, and assessment of cardiovascular disease risk factors. On registration, they were not suffering with acute or evident heart failure or acute inflammation whose serum.CRP is larger than 2 mg/dl. They were also not suffering with acute myocardial infarction or cerebral infarction within 3 months. We followed patients up to 2.8 years. All participants had a clinical visit each year of the study period, and their laboratory data were determined at each of these visits. We had telephone contact with the patients who could not have clinical visit, or their physicians.

Measurement

We measured fasting serum or plasma levels of biochemical products including lipids and plasma levels of neurohumoral factors and cytokines. Levels of general biochemical products were measured at SRL Laboratories, Tokyo, on an automated sequential multiple analyzer. Samples for the assay of plasma norepinephrine (NE), angiotensin-II, BNP, NO_x, cGMP, IL-6, and TNF-α levels were transferred to chilled disposable tubes containing EDTA-2Na. The blood samples were immediately placed on ice and centrifuged at −4 °C, and aliquots of plasma were immediately stored at −80 °C until assay. BNP levels were measured with a specific radioimmunoassay. NE levels were measured by HPLC. NO_x levels were measured using an NO detector-HPLC system (ENO10; Eicom Co., Kyoto, Japan) [22]. cGMP concentration was determined using a specific radioimmunoassay method (RPN226; Amersham, Buckinghamshire, England) [23]. Angiotensin-II levels were measured by radioimmunoassay. Both IL-6 and TNF-α measurements were performed using a commercially available radioimmunoassay kit (Quantikine HS; R&D Systems, Minneapolis, MN). Hypertension was defined as systolic BP ⩾ 140 mmHg, or diastolic BP ⩾ 90 mmHG or antihypertensive drugs were prescribed. Hyperlipidemia was defined as follows. Total cholesterol ⩾ 220 mg/dl or LDL cholesterol (total cholesterol − HDL cholesterol − triglyceride/5) ⩾ 140 mg/dl) or anti-hyperlipidemic drugs were prescribed. Diabetes mellitus was defined as in American Diabetes Society Guidelines [24] (in brief, fasting blood glucose ⩾126 mg/dl or hemoglobin A1C ⩾ 6.5 g/dl). Previously diagnosed hypertension, hyperlipidemia or diabetes were also included.

Statistical analysis

The results are presented as means ± SD. Values of $P < .05$ were considered to indicate statistical significance in all analyses. All statistical analyses were performed using Stat View software (SAS Institute Inc., Cary, NC). Characteristics of the survivors and the deceased subjects were compared using Mann–Whitney's *U*-test. Characteristics that were significantly different between the survivors and deceased by Mann–Whitney's *U*-test were further subjected to inherent multiple logistic regression analysis. As a result, adjusted odds ratios were calculated. Survival curves were calculated by the Kaplan–Meier method.

Results

Clinical characteristics

Table 1 shows the baseline characteristics of patients. There were no significant differences in age or coronary risk factors among the situations where the patients were