Estradiol to testosterone ratio in metabolic syndrome men aged started 40 years above

R Kusuma[1,5*], Y Siregar[1,2] and Mardianto[3,4]

[1]Post Graduate Program of Biomedical science, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Biochemistry, Faculty of medicine, Universitas Sumatera Utara, Medan, Indonesia
[3]Department of Internal Medicine, Faculty of medicine, Universitas Sumatera Utara, Medan, Indonesia
[4]Division of Endocrine & Metabolism, RSUP H.Adam Malik, Medan, Indonesia
[*]Corresponding author: ritkus.lit@gmail.com

Abstract. Disruption of adipose tissue, an endocrine organ, could turn out into the so-calledmetabolic syndrome. Aging men with lowering testosterone were related to metabolic syndrome and excessive aromatase activity in adipose tissue would increase estradiol level. This study hypothesized that estradiol to testosterone ratio is increasedin aging, metabolic syndrome men.A total of 52 men were randomly recruited for this study. A blood samplewas drawn before 11.00 AM after 10 hoursof overnight fasting, then aliquot serum kept in -20℃ pending the research. Subjects were divided evenly into the metabolic syndrome and nonmetabolicsyndrome group. The hormonal assaywas measured on the day of research. Then examined with student t-test. Estradiol level in metabolic syndrome group was increased, but insignificant differ to the other group. Testosterone level decreased and significantly different between groups. In conclusion, estradiol to testosterone ratio was increased in themetabolic syndrome group but insignificant.

1. Introduction

Nowadays, sedentary lifestyle with excessive caloric intake and overwhelming fast food had increased prevalence of overweight and adiposity in population. Consequently, emerged complex pathological disruption such as accumulation of visceral adipose tissue, dyslipidemia, insulin resistance and hypertension, with the so-called metabolic syndrome.[1]

Pathogenesis of metabolic syndrome and its components were complex, with the main causes are central obesity and insulin resistance. In addition to central obesity, fat distribution in the body such as visceral abdominal adipose tissue or intramuscular adipose tissue, known as ectopic fat, was related to the higher prevalence of metabolic syndrome in aging men,especially whose body weight was normal.[2]

Low total testosterone and clinical androgen deficiency are associated with increased risk of developing metabolic syndrome over time, particularly in overweight, middle-aged men.[3] The decline intestosterone levels is accompanied by a slight decline in estradiol levels, resulting in an increase of estradiol to testosteroneratio.[4] Previous studies have found that there has apositive association between estradiol level in elderly men with inflammation, stroke,and diabetes.[4] The

InCHIANTI study showed, in older men high estradiol is independently associated with metabolic syndrome.[5]

The prevalence of metabolic syndrome increases withage,and it is more common in men than women.[6] All across Asia, the number of elderly people is expected to grow dramatically. As a whole, the populationwill increase by 314 percent from 207 million in 2000 to 857million in 2050. Indonesia population data showed, there will be an increment of estimated population proportion age 65 years above from 4.9 % to 8.1% since the year 2005 to 2025. In mostcountries of Asia, as in the rest of the world, older women outnumbered older men, particularly in the elderly age groups.[7,8]

Metabolic syndrome increases with age and is more common in men than women in elderly. TO be able to improve quality of life and prevent early death of aging men it is important to determine the exact pattern of sex hormone changes with age. Because of the central role these hormones play in etiology, diagnosis, and management of several diseases. It has been proposed that the relative increase of circulating levels of estradiol derived from the conversion of testosterone into estradiol by aromatase activation in the adipose tissue, inhibits the hypothalamic-pituitary unit leading to the vicious cycle of "obese estrogenic hypogonadism."[5] This study tested the hypothesis that estradiol to testosterone ratio is increased in aging metabolic syndrome men compare to the nonmetabolic syndrome men.

2. Methods

This is a cross-sectional analytical, descriptive study conducted since June to August 2012, to all apparently healthy men age started from 40 years above (40-78 years) who did health screening at Spectrum clinical laboratory Medan, Indonesia. All participants were given and signed written informed consent before enrollment in the study and the Ethical Clearance Committee Review Board of Medical Faculty Universitas Sumatera Utara Medan, following the Declaration of Helsinki, approved the study. Participants were screened initially with a questionnaire detailing their medical history, smoking status, and concomitant medications. The inclusion criteria for the metabolic syndrome group referred to the Joint Scientific Statement 2009. So that the presence of any 3 of 5 risk factors constitutes a diagnosis of metabolic syndrome [9], i.e. elevated waist circumference ≥ 90 cm in males; which cutpoint based on Asia Pacific Population specific definitions of obesity for men, elevated triglycerides ≥ 150 mg/dl (drug treatment for elevated triglycerides is analternate indicator), reduced HDL-C ≤ 40 mg/dl in males; ≤ 50 mg/dl in females (drug treatment for reduced HDL-C is an alternate indicator), elevated blood pressure Systolic ≥130 and/or diastolic≥85 mmHg (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator), elevated fasting glucose≥100 mg/dL (drug treatment of elevated glucose is analternate indicator). Subject who had cardiovascular disease, malignancy, prostate disease, hepatitis, smoking, and alcoholic was excluded. Those who were ever or being treated with estrogen or testosterone supplementation, aromatase inhibitor in this recent 3 months were excluded as well. Total 52 men were selected by consecutive sampling technique and divided into the metabolic syndrome and the non-metabolic syndrome group each for 26 persons.

2.1.Components of Metabolic Syndrome

Weight and height were measured using standard techniques.Waist circumference was measuredwith a measurement tape position around the waist midway between the lower rib marginand the iliac crest with participants in a standing positionwithout heavy outer garments,while the examiner remains on the participant's right side. The tape sits parallel to the floor and lies snug but does not compress the skin. Take the measurement to the nearest 0.1 cm at the end of the normal expiration.[10] Baseline blood pressure was recorded using a standard mercury sphygmomanometer and stethoscope.All blood pressure measurements were performed with the participant in a seated position for at least 5 minutes and feet rest comfortably on the floor, on three occasions separated by intervals of 2 minutes,and the average of the last two measureswas used in the analysis.

2.2.Blood Assays
A minimally 10 hours overnight fasting blood samples were drawn between 07.30 and 11.00 AM. Serum samples were obtained by centrifugationand parts were used to measure the component of metabolic syndrome,i.e., fasting blood glucose level, HDL-C level,and triglyceride level. The aliquot serum immediatelystoredwith thelabeled identity of each participant at - 20℃ pending further hormonal assay. On the day of analysis, stored frozen samples were thawed, then all together were measured total estradiol and testosterone level by Microparticle Enzyme Immunoassay (MEIA) technology, using Abbot AxSYM SYSTEM ESTRADIOL and TESTOSTERONE ASSAY. Estradiol and testosterone level measurement was recorded in pg/ml, and ng/ml respectively.

2.3.Statistical Analysis
This was a numerical analytic descriptive statistic, one-way hypothesis study. Firstly, data were tested analytically with Shapiro-Wilk test because of the small sample size (≤ 50), to determine the data distributed normally or not. Distributed normally data group was tested with unpaired t-test, while undistributed normally data group was tested with Mann-Whitney test. P value < 0.05 was stated significant. Data were analyzed with SPSS software (Statistic Package for Social Science). Results of the study elaborated in tables and stated in mean ± s.d (range) (Table 1).

3. Results

Table 1. Characteristic of the study subjects group based on metabolic syndrome or not.

	Population Characteristic		
	MS (n = 26)	**Non MS (n = 26)**	**P[a]**
Age (year)	55 ± 11.39 (41-78)	56 ± 10.50 (40-74)	0.3235
Weight (kg)	82 ± 14.72 (58-113)	69 ± 9.51 (47-90)	0.0005[a]
BMI (kg/m^2)	28 ± 4.20 (21.9-37.3)	24.8 ± 2.70 (19.3-31.9)	0.0005[a]
WC (cm)	93.4 ± 10.4 (64-116.5)	83.5 ± 7.7 (63.5-100.5)	0.000[a]
Syst BP (mmHg)	127 ± 14.37 (100-160)	120 ± 20.39 (100-200)	0.008[a]
Diast BP(mmHg)	86 ± 6.17 (80-100)	81 ± 7.31 (70-100)	0.003[a]
Fasting Gluc (mg/dl)	102 ± 18.29 (66-144)	86 ± 10.10 (65-112)	0.000[a]
HDL-C (mg/dl)	40 ± 9.59 (27-65)	44 ± 8.33 (31-62)	0.033[a]
TG (mg/dl)	132 ± 50.15 (59-245)	89 ± 30.76 (40-161)	0.000[a]
Tot E2 (pg/ml)	34 ± 19.68 (2-93)	28 ± 11.31 (0-51)	0.357
Tot T (ng/ml)	4.91 ± 1.77 (1.84-9.29)	5.65 ± 1.22 (3.83-8.35)	0.043[a]
Ratio E2/T (.10^{-3})	7.41 ± 4.98 (0.93-22.68)	5.12 ± 2.19 (0.00-9.92)	0.076

MS (Metabolic Syndrome), BMI (Body Mass Index), WC (Waist Circumference), Syst BP (Systolic Blood Pressure), Digest BP (Diastolic Blood Pressure), Fasting Gluc (Fasting Glucose Level), HDL (high density lipoprotein Cholesterol), TG (triglyceride), Tot E2 (total estradiol), Tot T (total testosterone)
[s]ignificance test <0.05

Table 1 shows the general characteristics of the study population.Overall52 participants divided into two groups, the metabolic syndrome,andnosyndrome group, 26 persons each group. There were significantly different between the metabolic syndrome to the non metabolic syndrome group (P < 0.05) for the risk factor such as body weight, body mass index, and all the metabolic syndrome component,i.e. waist circumference, blood pressure, fasting glucose, HDL-C (high density lipoprotein cholesterol), and triglyceride level.

3.1. Hormone level related to metabolic syndrome
By the unpaired t-test analysis, mean total testosterone level decreased significantly (P = 0.04) between the metabolic syndrome group to the nonmetabolic syndrome group. Whereas by the Mann-Whitney analysis, mean estradiol level did not increase significantly nor the ratio of estradiol to

testosterone (P = 0.357 and P = 0.076 respectively) between the metabolic syndrome group to the nonmetabolic syndrome group.

4. Discussion

In thiscross-sectional descriptive analytic study of total 52 men age from 40 years above showed that mean total testosterone level decreased significantly between the metabolic syndrome group to the nonmetabolic syndrome group. Whereas mean estradiol level did not increased significantly nor the ratio of estradiol to testosterone between the metabolic syndrome group to the nation syndrome group.

This study tested aging men started from 40 years of age, as men age beyond 40 years, they experience adecline in serum testosterone. For the hormonal assay, this study measured total testosterone and total estradiol. Testosterone known to be important an objective parameterby which to examine in ageing men.The ageing processisassociated with a decline in lean body mass andan increase in fat mass.[11] Obesity and aging are both associated with chronic, low-grade inflammation and insulin resistance, increased local and circulating proinflammatory, chemotactic, and procoagulant proteins, and ectopic lipid deposition with lipotoxicity. Fat is redistributed among different fat depots over time, especially during and aftermiddle age, that fat redistributes from subcutaneous to intraabdominal visceral depots. Visceral fat enlargement is more strongly associated metabolic disease than generalized obesity, especially in old age. Subcutaneous fat expansion in obesity may actually be protective.[12] Waist circumference (WC) was used to determine obesity in this study, not body mass index (BMI).As WC is the anthropometric index that most uniformly predicts the distribution of adipose tissue among several fat compartments in the abdominal region, there apparently being little value in measuring waist to hip ratio (WHR) or BMI.[13]

Testosterone in men 95% produced by testes, is necessary for sexual differentiation and reproductive function, also regulates gene expression in most extra-genital tissues, including muscle and bone, and the immune system. A young adult man generally produces 3 to 10 mg of testosterone daily (300to 1000 ng/dL).[14] Total serum testosterone consists of freetestosterone (2%−3%), testosterone bound to sex hormone binding globulin (SHBG) (45%) and testosterone bound toother proteins (mainly albumin −50%). Testosterone binds only loosely to albumin and so this testosteroneas well as free testosterone is available to tissues andis termed bioavailable testosterone. Testosterone bound toSHBG is tightly bound and is biologically inactive.[11] There is diurnal variation in serumtestosterone levels with peak levels seen in the morning followingsleep, which can be maintained into the seventh decade. Samples should always be taken in themorning before 11 AM to allow for standardization.[11] The diurnal testosterone rhythm is blunted as men grow older.[14]

In males, estrogens mainly derive from circulating androgens. The key step in estrogen biosynthesis is the aromatization of the C19 androgens, testosterone and androstenedione, to form estradiol (E2) and estrone (E1), respectively. This step is under the control of the aromatase enzyme. In men, estrogen have physiologic action in reproductive system, metabolism and bone health.[15] In adult men, the normal range of serum estradiol is 18-40 pg/mL. Approximately 50 μg of estradiol are produced daily: about 5-10μg in the testis (10 to 20%) and the remaining 40-45 μg (80 to 90%) in peripheral tissues (adipose tissue, muscle, breast, brain liver and bone) in which the aromatase enzyme is expressed.[15] No diurnal variation was detected for estradiol level in men.[15] Most of the circulating estradiol in men is loosely bound to albumin or is unbound, and only about 20% is bound to SHBG. Because estradiol binds to SHBG with lower affinity than does testosterone, the serum level of SHBG was not predicted to influence substantially the actions of estradiol.[14]

Several mechanisms have been suggested for the causal relationship between low testosterone and abdominal obesity. Activation of the lipoprotein lipase and lipolysis may explain the effect of testosterone on adipose tissue. Conversely, it has been reported that men with metabolic syndrome are prone to hypogonadism. This might be due to elevated leptin levels that interfere with gonadotropin-stimulated androgen production and to increased aromatase activity in adipocyte that leads to higher circulating estradiol and suppression of testosterone production by negative feedback on the

hypothalamic-pituitary-gonadotropin (HPG) axis. These findings suggest a bi-directional causal relationship between low testosterone and adiposity (Figure 1).[16]
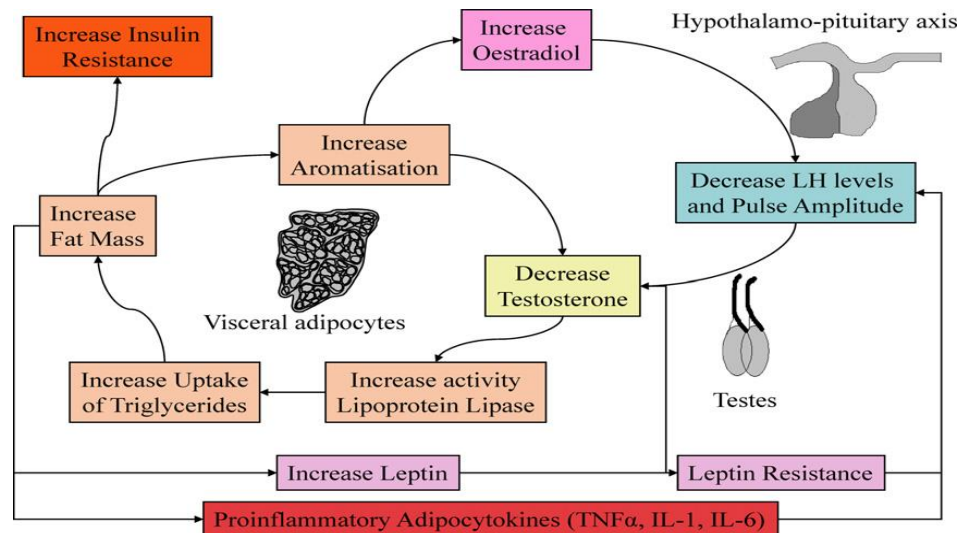

Figure 1. Hypogonadalobesity-adipocytokine cycle [16].

Total testosterone level affected by sex hormone binding globulin (SHBG) level.[14] And single nucleotide polymorphisms in the SHBG gene appear to influence the affinity of SHBG for testosterone and affect the level in plasma.[14] Polymorphism of the CYP19 gene (rs2470152) associated with higher estradiol level in men.[17] The longer*AR*CAGrepeats also had higher testosterone and estradiol levels significantly.[18]

This study found that estradiol to testosterone ratio in aging metabolic syndrome men was increased, but insignificant compared to the nonmetabolic syndrome group (P = 0.076). Limitation in this study was using quite a large effect size so that the sample size was small. This study also not excluded subject with diabetes on medication. Not all the metabolic syndrome group subjects had abdominal adiposity (WC ≥ 90 cm; cutpoint for Asia Pacific men population). In addition, SHBG level, medication,and several gene polymorphisms could affect the estradiol and testosterone plasma level. The strength of this studythat all subjects were well selected with consecutive sampling and refer to the inclusion and exclusion criteria. Blood samples were drawn before 11.00 AM after fasting overnight to anticipate the diurnal fluctuation of the testosterone level. The implication in clinical practice of this study, to get a more appropriate treatment management for metabolic syndrome in men,especially the middle-aged and aging men. So that to prevent premature death of men with metabolic syndrome.

In conclusion, estradiol to testosterone ratio in metabolic syndrome aging men was increased, but insignificant statistically compared to the nonmetabolic syndrome group. Further research was needed with larger sample size, included to measure the SHBG and albumin level and inclusion criteria of thesubject with waist circumference determination of the metabolic syndrome men.

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