International Journal of Pharma and Bio Sciences V1(2)2010

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

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ABSTRACT

Background

Aging is associated with changes in physical characteristics and decline of many physiological functions. There are several conflicting and inconsistent data reported on the relationship between individual antioxidants and aging. Hence, the present study was designed to study the significance of malondialdehyde : total antioxidant capacity (MDA:TAC) ratio as novel indicator of oxidative stress in aging.

Methods

In this study, we determined the serum MDA and TAC on healthy non-obese males . Healthy subjects were classified into 3 groups, elderly (>50 yrs), middle-aged (35-49 yrs) and young group (20-34 yrs).

Results

MDA:TAC ratio increased with advancing age. Oxidative stress correlated with decrease in waist-to-hip ratio.

Conclusion

These results are suggestive of oxidative stress with advancing age. MDA:TAC ratio can be a useful indicator to monitor and optimize antioxidant therapy which may reduce morbidity and perhaps increase the healthy, useful life span of an individual.

KEY WORDS

Aging, lipid peroxidation, malondialdehyde, total antioxidant capacity, waist-to-hip ratio, body mass index.

INTRODUCTION

Aging is a process of irreversible physiological and morphological changes associated an increasing susceptibility to disease. Oxygen free radical mediated cell damages are implicated in the aging process [1]. Oxidative damage to DNA, proteins and lipids induced by the overproduction of reactive oxygen species (ROS) accumulate with age and contributes to degenerative diseases and the aging phenomenon by disrupting cellular homeostasis. On

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

the other hand, the human body generally has antioxidant system which plays an important role in the suppression of ROS overproduction and protects cells from oxidative stress. Antioxidant system is comprised of endogenous compounds (bilirubin, uric acid, superoxide dismutases, catalase, glutathione peroxidase etc.) and exogenous compounds (carotenoids, tocopherols, ascorbate, bioflavonoids etc) [2]. The determination of malondialdehyde (MDA) is used for monitoring lipid peroxidation in biological samples. Although the concentration of plasma antioxidant components can be measured individually, these measurements may be time- and cost-consuming and labour intensive. In addition, it may not accurately reflect the total antioxidant status. Total antioxidant capacity (TAC) considers the cumulative effect of all antioxidants present in blood and body fluids [3]. There are several conflicting and inconsistent data reported on the relationship between oxidative stress and aging [4]. Hence, the present study was designed to study the significance of MDA : TAC (novel indicators of oxidative stress) ratio in healthy non-obese males of different age groups, which may be useful to optimize and monitor the antioxidant therapy in aging.

MATERIALS AND METHODS

A cross-sectional and comparative study was conducted at Sri Siddhartha Medical College and Research Centre, Tumkur, in a sample of 100 healthy men classified into 3 groups, young group (20-34 yrs, n=32), middle-aged (35-49 yrs, n=33) and elderly (>50 yrs, n=35). All individuals were healthy (**without** chronic illness / cardiovascular risk factors / diabetes / vascular / renal / liver disease / respiratory disease / malignancy), well nourished (their caloric intake were between 2000-2500 kcal / day, their alimentation had all nutrient requirements with carbohydrates, lipids, proteins, minerals and vitamins consistent with recommended dietary allowance measured by 24 hr dietary recalls and their serum albumin were > 3.5g / dl), nonsmokers, non-alcoholics and without any antioxidant supplementation or other drug intake, to avoid the possible confounding effect of such factors on lipid peroxidation. The physical activity was similar between all these three groups studied. The study was conducted after informed consent was obtained from them and the study was approved by the ethical committee of the institution. Anthropometric measurements including height, body weight, body mass index (BMI) were carried out among the study group. BMI was calculated from the formula, weight (kg) / height (meters)^2. Waist-to-hip ratio (W/H) was calculated by dividing waist circumference by hip circumference. It gives an indication about the distribution of body fat, thus defining the central or visceral obesity. Normal reference value for W/H is <0.85 in females and <0.95 in males.

Under aseptic precautions 5 ml of fasting venous blood samples were collected in plain vacutainers. Clotted blood was subjected to centrifugation within 3 hrs of collection at 3000 rpm for 10 minutes. The clear serum was separated and used for measuring MDA and ferric reducing antioxidant power (FRAP) levels. All the chemicals used were of highest analytical grade available in India. Lipid peroxidation was measured by serum MDA estimation according to the colorimetric method of Satoh.k [5]. Lipoproteins are precipitated from the specimen by adding trichloroacetic acid. 0.05M sulphuric acid and 0.67% thiobarbituric acid (TBA) in 2M sodium sulphate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling waterbath for 30 minutes. The resulting chromogen is extracted in n-butanol, which is measured colorimetrically at 530 nm. Total antioxidant capacity was measured by FRAP assay according to the method of Benzie.F.F. and J.J.Strain [6]. At low pH, when a ferric tripyridyltriazine (Fe ^{III}-TPTZ) complex is reduced to the ferrous (Fe ^{II}) form, an intense blue colour with an absorption maximum at 593 nm develops.

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (M ± SD). Statistical comparisons were done using student't' test and one way ANOVA test. 'p' value < 0.05 was considered statistically significant. Pearson's correlation coefficients were determined between the measured parameters at 5% level of significance.

RESULTS

Significant increase in MDA values progressing with age was observed. TAC showed significantly decreased values progressing with age. Mean BMI was increased in elderly group compared to young and middle age groups. Though, W/H ratio was decreasing progressively with increasing age, there was no significant difference in W/H ratio between the three groups. [TABLE 1]. MDA:TAC ratio progressively increased with increasing age. [FIGURE 1]. In the middle-age group, there were significant negative correlations between MDA and W/H and between TAC and MDA. In the elderly age group, there was a significant positive correlation between TAC and W/H , significant negative correlations between MDA and W/H and between TAC and MDA. [TABLE 2].

TABLE 1.

COMPARISON OF THE MEASURED PARAMETERS IN YOUNG , MIDDLE AND ELDERLY AGE GROUPS.

Parameter	YOUNG AGE 20-34 YRS (n=32) Mean ± S.D	MIDDLE AGE 35-49 YRS (n=33) Mean ± S.D	ELDERLY AGE >50 YRS (n=35) Mean ± S.D	p value
MDA (nmol/ml)	0.78 ± 0.12	0.99 ± 0.11	1.2 ± 0.11	<0.05*
FRAP (µmol/L)	1215.15 ± 55.57	1103.64 ± 86.52	935.59 ± 90.19	<0.05*
BMI	21.04 ± 1.75	23.25 ± 4.47	31.2 ± 0.11	<0.05*
W/H	0.78±0.06	0.88±0.08	0.91±0.02	<0.05*
MDA:TAC	0.0006	0.0008	0.0012	<0.05*

** p< 0.05 – Significant.*

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

FIGURE .1

MDA: TAC RATIO IN DIFFERENT AGE GROUPS OF HEALTHY NORMAL MALES

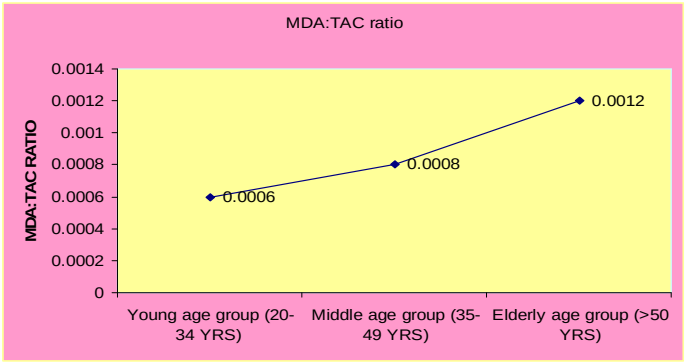

TABLE 2.

CORRELATION BETWEEN THE OXIDATIVE STRESS MARKERS AND ANTHROPOMETRIC PARAMETERS IN DIFFERENT AGE GROUPS.

2A. MIDDLE AGE GROUP

PARAMETERS	R VALUE	p VALUE
MDA and W/H	0.01	<0.05*
MDA and TAC	- 0.01	<0.05*

*p< 0.05 – Significant.

2B. ELDERLY AGE GROUP

PARAMETERS	R VALUE	p VALUE
MDA and W/H	0.01	<0.05*
TAC and W/H	- 0.01	<0.05*
MDA and TAC	- 0.01	<0.05*

*p<0.05 – Significant.

DISCUSSION

Aging is associated with changes in physical characteristics and decline of many physiological functions. The excessive production of free radicals in the organism, and the imbalance between the concentrations of these and the antioxidant defenses may be related to aging [7,8]. Oxidative stress results from the imbalance between oxidative and antioxidative mechanisms with increased levels of pro-oxidants and depletion of antioxidants leading to tissue damage. Direct measurement of ROS and free radicals in a standard laboratory is difficult owing to their biochemical instability. The main component of ROS is hydroperoxides. Despite fair oxidant power, hydroperoxides in the blood are relatively stable compared to its parent free radicals, and therefore, the level can be detected. Hydroperoxides cause cell death and tissue damage. MDA is a three carbon, low molecular weight aldehyde that can be

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

produced from free radical attack on polyunsaturated fatty acids of biological membranes. The determination of MDA is used for monitoring lipid peroxidation in biological samples [9,10]. Few studies have reported that there is no significant age-related progressive increase of ROS when there are no pro-oxidant factors such as smoking [11,12]. In the present study, we found serum MDA levels were significantly higher in the group of elderly persons, as compared with the younger and middle age groups.

Several studies have produced conflicting results regarding antioxidant status in aging. Although, few studies suggest decrease in anti-oxidant system with advancing age, several other studies reported that antioxidant activity remains unchanged with aging. FRAP assay is considered as a useful indicator of the system's ability to regulate the damage due to ROS and thus, a novel method of assessing total antioxidant capacity [6,13], as the individual antioxidant components may not fully reflect the protective efficiency of blood, probably because of interactions that occur in vivo among different antioxidant compounds. In the present study, TAC showed significantly decreased values progressing with age.

Although previous studies have focussed on either increase in lipid peroxidation markers like MDA alone or decreased levels of antioxidant markers like superoxide dismutase, vitamin C, vitamin E etc, this study emphasizes the fact that imbalance between oxidant:antioxidant ratio is the crucial factor to determine the oxidative stress. There can be compensatory mechanisms to overcome the lipid peroxidation (increased MDA levels) by increasing the antioxidants in vivo which can maintain the normal oxidant:antioxidant ratio (without oxidative stress). Also, increase in only enzymatic or non-enzymatic antioxidants alone may not reflect the total antioxidant status of an individual. Our study is unique and first of its kind to make an attempt to establish the significance of MDA:TAC ratio as a new marker of oxidative stress rather than concentrating on increased MDA alone or decreased levels of individual antioxidants.

BMI is a measure of relative weight, which correlates highly with percentage of body fat and is largely independent of height. BMI does not provide any indication regarding distribution of fat in the body [14]. Although the importance of smoking and preexisting illness as confounders of the relation between body-mass index and mortality has been challenged, we thought it prudent to avoid potential confounding by these factors. Nevertheless, more older subjects than younger ones may have had reduced weight at base line. W/H is a preferable index for visceral adiposity than other common measures of obesity, such as BMI or waist circumference alone [15]. Studies that compare the relations between the body-mass index (the weight in kilograms divided by the square of the height in meters) and aging are rare. The effect of age on optimal body weight is controversial [16]. In the present study, decrease in BMI had a positive relationship with oxidative stress, but MDA and TAC did not vary with body mass index. In contrast, significant positive correlation of W/H with increasing MDA levels and significant negative correlation between W/H and TAC levels with progressing age suggested oxidative stress.

CONCLUSION

Our findings on non-obese healthy males of different age groups suggest that there might be an association between oxidative stress and the age progression. The possibility of counteracting oxidative stress by a pool of proper antioxidants plus an appropriate diet, may have real health benefit to reduce morbidity and perhaps increase the healthy, useful life span of an individual. Future studies will need to address targeting therapy to specific tissues and finding appropriate markers for functional

AGE RELATED CHANGES IN MALONDIALDEHYDE: TOTAL ANTIOXIDANT CAPACITY RATIO – A NOVEL MARKER OF OXIDATIVE STRESS.

consequences of the attenuation of oxidative stress in aging.

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