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RESEARCH ARTICLE

A cross-sectional study of serum level of malondialdehyde in type 2 diabetes mellitus individuals of rural population of North Karnataka

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ABSTRACT

Background: Oxidative stress is not only involved in the pathogenesis of many chronic diseases like diabetes mellitus (DM) but also in various physiological processes like aging. In this process, highly reactive free radicals are formed. These free radicals react with lipids and carry out lipid peroxidation, which result in the formation of aldehyde products like malondialdehyde (MDA). The serum level of MDA can serve as an important indicator of oxidative stress. This study helps to assess differences in MDA level as a biomarker of oxidative stress in healthy and type 2 DM (T2DM) patients. **Aims and Objectives:** This study aims to investigate the serum MDA level in T2DM patients and its association with other risk factors such as obesity, diet, and duration of diabetes in T2DM patients. **Materials and Methods:** The present cross-sectional study was conducted in 93 T2DM patients and 93 healthy individuals. Serum levels of MDA are the most commonly used markers of this process which was measured as thiobarbituric acid reactive substances (TBARS). The MDA-TBA adduct so formed has a colorimetric measurement at 532 nm on spectrophotometer. **Results:** The MDA concentrations of healthy controls and T2DM patients were 0.931 and 2.684 μmol/l, respectively. The serum MDA concentration in T2DM patients was significantly elevated ($P < 0.001$) compared with healthy individuals. MDA levels were significantly correlated ($P < 0.001$) with conventional risk factors such as diet, truncal obesity, and duration of diabetes in T2DM patients. **Conclusion:** Serum level of MDA was significantly increased in T2DM patients. It may be a good marker of oxidative stress that is involved in various complications of T2DM. Elevated MDA levels are also associated with conventional risk factors such as diet, truncal obesity, and duration of diabetes.

KEY WORDS: Lipid Peroxidation; Malondialdehyde; Oxidative Stress; Type 2 Diabetes Mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is recognized as one of the major causes of morbidity and mortality in the world. T2DM has been shown to increase free radical activity; in fact, it's close association with the development of insulin resistance and T2DM is also been investigated.[1,2] This increased free radical activity in T2DM results in accumulation of lipid peroxidation products which are responsible for higher prevalence of atherosclerotic and cardiovascular complications.[3-5] Various mechanisms that are involved in the formation of free radicals in T2DM are increased non-enzymatic and auto-oxidative glycosylation of proteins, changes in energy metabolism, leading to metabolic stress, and, in turn, increased levels of inflammatory mediators.[6,7]

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Lipid peroxidation is an autocatalytic free radical-mediated process, wherein lipid hydroperoxides are formed as a

result of action of free radicals on polyunsaturated fatty acids (PUFAs) in cell membranes.[8] In this process, free radicals attack double bonds of PUFAs. Such a reaction leads to the formation of by-products such as conjugated dienes and malondialdehyde (MDA). As a result, the serum level of MDA is increased in T2DM patients.[8] MDA thus generated as a relatively stable end product of lipid peroxidation of PUFAs.[8-10] This free radical-mediated lipid peroxidation is not only been investigated in disease processes such as atherosclerosis, Alzheimer's disease, and cancer but also in normal physiological process like aging and also found to be causatively involved in the process.[11-15] Thus, serum MDA can be used as biomarker of lipid peroxidation (oxidative stress) which is an indicator of free radical damage.

MDA is a three-carbon dialdehyde that can exist in various forms in an aqueous solution. This method is most widely used for the estimation of MDA with thiobarbituric acid (TBA) when heated under acidic conditions.[16] The TBA can react with a number of chemical species such as nucleic acids, amino acid, proteins, phospholipids, and aldehydes. One molecule of MDA reacts with two molecules of TBA to form a stable pink to red color fluorescent 1:2 MDA:TBA adduct that absorbs maximally at 532 nm in a spectrophotometer. These substances are termed as TBA reactive substances (TBARS).[17,10] Thus, oxidative stress was assessed by quantifying MDA as TBA reactivity. Hence, the current study is carried out to assess the levels of MDA (marker of oxidative stress) in T2DM patients and its association with risk factors such as diet, truncal obesity, and duration of disease in patients with T2DM individuals of rural population of North Karnataka.

Objective

The objective of this study was to assess the serum level of MDA in T2DM individuals of rural population of North Karnataka and its association with risk factors in patients with T2DM.

MATERIALS AND METHODS

Study Setting

A community-based cross-sectional study was carried out in a Primary Health Center (PHC) area of Handignur, of North Karnataka. A total of 662 participants (with response rate of 91.54%) were screened for T2DM in Handignur PHC, of which 93 (14%) participants were diagnosed as T2DM (55 males and 38 females).

Inclusion Criteria

Participants with age above 30 years with symptoms of T2DM and known cases of T2DM as well with fasting blood sugar >126 mg/dl were included in the study.

Exclusion Criteria

Participants with type 1 DM, with cognitive, neurological, psychological, and endocrinal disorder, pregnant women were excluded from the study.

The study was approved by the Institutional Ethics Committee on human subject research. After the selection, participants were briefed about the nature of the study and written informed consent was obtained.

Sample Size

The sample size was calculated based on reported prevalence of DM in rural areas (lowest being 3.8%) that are geographically and socioculturally similar to the study area. Considering absolute error of 1% with 95% confidence level (inflation factor for cluster sampling considered as 2), the sample size was estimated to be 662 as screening population.

Family History of Diabetes

Detailed family history of T2DM patients was taken. The family history of T2DM was verified by two ways, either by blood glucose measurement of the parents or by other evidence such as physician report, consumption of drugs, and diet modifications.[18]

Diet

Depending on the type of food preferred participants were classified into vegetarian and mixed type.

Duration of type 2 diabetes

Duration of diabetes was classified as newly diagnosed and 1–5 years as short-term diabetes, and 6 years and above as long-term diabetes.[19]

Anthropometrical Measurements

Anthropometric measurements included height, weight, waist circumference, hip circumference, and waist–hip ratio.

Weight (kg)

Weight was recorded using a standard weighing scale (Krups weighing scale) that was kept on firm horizontal surface.

Height (cm)

Height was measured by commercial stadiometer. The participant was made to stand erect with barefoot on the floorboard of the stadiometer with his or her back to the

vertical backboard of the stadiometer. The heels of the feet are placed together with both heels touching the base of the vertical board. The buttocks, scapulae, and head are positioned in contact with the vertical backboard.

Body mass index (BMI)

BMI was recorded using Quetelet's equation (Weight [kg]/height [m^2]), classification of BMI: ≤18.9 kg/m^2 – underweight, 19–24.9 kg/m^2 – normal, 25–29.9 kg/m^2 – pre-obese (overweight), and ≥30 kg/m^2 – obese.[18]

Waist circumference (cm)

Waist circumference was measured at the midpoint between the costal margin and iliac crest using a non-stretchable measuring tape at the end of normal expiration with the subject standing erect in relaxed position.[18]

Hip circumference (cm)

Hip circumference was measured at the level of greater trochanters (widest position of hip) with a non-stretchable measuring tape, while the subject was standing with the arms by side and feet together.[18]

Waist–hip Ratio

Waist–hip ratio was calculated as the ratio of waist circumference and hip circumference.

Central Obesity

Central/abdominal obesity was considered to be present when waist circumference ≥90 cm in males and ≥80 cm in females.[18,19]

Truncal Obesity

Waist–hip ratio of >1.0 for males and >0.85 for females was defined as truncal obesity.[18,19]

Blood Pressure Measurement (mmHg)

Blood pressure was measured on the left arm in sitting posture, with the subject in a relaxed state. Standard mercury sphygmomanometer (diamond deluxe blood pressure [BP] apparatus, Pune, India) with adult size cuff was used.

Biochemical Screening Method for T2DM

Fasting blood glucose (FBS) measurements

Blood glucose estimation was done for all participants irrespective of whether they had diabetes or not. The subjects were asked to be on overnight fasting (for 8 h). Next morning after confirming fasting, blood glucose was measured using a standard digital glucometer (Omni test Plus B-Brown, Germany). All those who had FBS more than 126 mg/dl were considered as diabetic as per the WHO criteria.

MDA Estimation

To 0.5 ml of the serum 0.5 ml of 30% trichloroacetic acid (Merck) was added and centrifuged at 3000 rpm for 5 min and supernatant was collected. Thereafter, 0.5 ml of supernatant was added to 0.5 ml of 1% TBA (Merck) in a boiling water bath for 30 min following which tubes were kept in an ice-cold water bath for 10 min. The resulting chromogen absorbance was determined at the wavelength of 532 nm at room temperature against blank reference. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3'-tetraethoxy propane. The extent of lipid peroxidation was expressed as MDA (μm/L) using a molar extinction coefficient for MDA of 1.56×10^5/m/cm.[20]

Statistical Analysis

Data analysis was done using the Software Package of the Social Sciences version 16 and involved quantitative variables summarized through mean and standard deviation. Differences were considered significant if $P < 0.05$ with confidence interval of 95%. Risk factors association with elevated MDA levels was analyzed using Chi-square test.

RESULTS

A total of 662 participants with ≥30 years of age meeting the eligibility criteria were enrolled in the study. Among the total, 606 were screened with response rate of 91.54% and data were analyzed.

Figure 1 shows screening population. A total of 93 T2DM patients and 93 healthy participants (186) (75 males and 111 females) with a mean age of 52.26 ± 8.84 years were recruited from Handignur PHC area and its subcenters for the study.

Table 1 shows the anthropometric data of both diabetic and non-diabetic population which shows that age, height, weight, and BMI were statistically significant. However, waist–hip ratio between the two groups was not statistically significant.

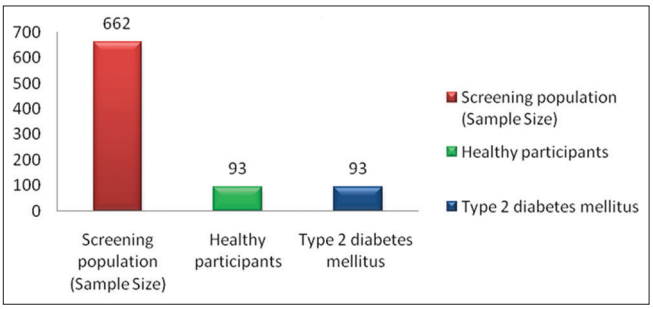

Figure 1: Screening population

Table 2 shows biochemical variables between diabetic and non-diabetic participants which show that fasting blood sugar between the two groups was significantly different ($P < 0.001$).

Table 1: Comparison of anthropometric variables between diabetic and non-diabetic participants

Variable	Category	Mean±SD	*P*
Age	Non-diabetic	52.01±8.8	0.002*
	T2DM	53.43±8.5	
Height	Non-diabetic	156.4±8.0	<0.001*
	T2DM	154.7±8.0	
Weight	Non-diabetic	59.6±7.9	<0.001*
	T2DM	67.27±8.5	
BMI	Non-diabetic	24.43±3.3	<0.001*
	T2DM	28.25±4.3	
Waist-hip ratio	Non-diabetic	0.88±0.06	0.075
	T2DM	0.88±0.07	

*P<0.05. SD: Standard deviation, T2DM: Type 2 diabetes mellitus

Table 2: Comparison of biochemical variables and blood pressure values between diabetic and non-diabetic participants

Variable	Category	Mean±SD	*P*
FBS	Non-diabetic	100.2±11.4	<0.001*
	T2DM	128.2±35.1	
MDA	Non-diabetic	0.91±0.35	<0.001*
	T2DM	2.55±1.40	
DBP	Non-diabetic	87.7±5.6	0.390
	T2DM	85.5±8.4	
SBP	Non-diabetic	125.4±10.5	<0.001*
	T2DM	129.0±13.2	

*P<0.05. FBS: Fasting blood glucose, MDA: Malondialdehyde, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, T2DM: Type 2 diabetes mellitus

Thus, they are classified into two groups as diabetic and non-diabetic depending on FBS values. The mean serum MDA level for non-diabetic participants was 0.91 ± 0.35 and that of diabetics was 2.55 ± 1.40 which was significantly higher in T2DM compared with non-diabetic individuals ($P < 0.001$). Table 2 also shows BP values of both the groups.

Table 3 shows association of type of diet, central and truncal obesity, and duration of diabetes with that of MDA levels among T2DM patients. Regarding association of diet and MDA levels shows that patients having mixed type of diet had higher risk of elevated MDA levels (oxidative stress) than patients having only vegetarian food, and the association was statistically significant ($P = 0.011$). Obesity, especially truncal obesity, was also found to be associated with higher risk of elevated MDA levels among T2DM. The association was statistically significant ($P = 0.001$). We also found that duration of diabetes was also associated with significantly higher risk of elevated MDA levels, especially those with >6 years of diabetes (long-term diabetes) than those with <6 years of diabetes, and the association was statistically significant ($P < 0.001$). In fact as the duration of diabetes advances the no. of patients with increased oxidative stress also increases.

DISCUSSION

Experimental and clinical studies show that oxidative stress does play a role in the pathogenesis of T2DM.[21,22] In the present study also, levels of MDA (as TBARS) were significantly elevated in T2DM patients. This finding is in line with earlier studies done in this context.[23,24] This serum MDA is proven to be associated with heightened oxidative stress.[23] This increased oxidative stress is due to increased free radical activity[1,2] which leads to a higher incidence of atherosclerotic and cardiovascular diseases.[25] Free radicals are formed disproportionately in DM by oxidative phosphorylation, glucose auto-oxidation, NADPH

Table 3: Association between diet, central obesity, truncal obesity and duration of diabetes with elevated MDA level in T2DM patients

Risk factors	Sub-category	Total number of T2DM (*n*=93) (%)	Number of patients with elevated MDA level (%)	X^2	df	*P* Value
Diet	Veg	24 (25.80)	5 (20.83)	6.201	1	0.011*
	Mixed	69 (74.19)	40 (66.9)			
Central obesity	No	25 (53.4)	5 (20)	0.011	1	0.522
	Yes	68 (46.6)	28 (48.11)			
Truncal obesity	No	36 (48.5)	17 (47.1)	3.252	1	0.001*
	Yes	57 (51.5)	51 (89.47)			
Duration of DM	Newly detected	20 (21)	5 (25)	4.223	4	<0.001*
	≤5 years	8 (8.6)	4 (50)			
	6-10	31 (33.3)	24 (77.41)			
	≥11	34 (36.5)	30 (88.2)			

*P<0.05. T2DM: Type 2 diabetes mellitus, MDA: Malondialdehyde

oxidase, lipooxygenase, cytochrome P_{450} monooxygenases and NOS enzymes, glucose degradation, and non-enzymatic glycation of proteins.[26] Subsequent oxidative degradation of glucose plays an important role in the development of complications such as atherosclerosis, heart disease, peripheral nerve damage, retinopathy, and cataract in T2DM patients.

In the present study, it was also observed that diet, duration of diabetes, and truncal obesity were significantly associated with elevated level of MDA (oxidative stress) in T2DM patients. The study conducted by Domínguez *et al*. showed that estimated level of MDA was higher with the earlier onset and prolonged duration of type 1 diabetes. This study also showed that these levels continue to rise during the course of the disease.[27] Nakhjavani *et al*. observed that elevated MDA level was significantly correlated to longer duration of T2DM than shorter duration.[28] As age also an important factor that is associated with oxidative stress along with DM, the association between duration of DM and MDA level remained significant even after adjustment was made for age.[28] Kaefer *et al*. also observed that serum MDA level was increased in T2DM patients than healthy individuals and this increased level was also associated with the duration of DM.[29]

In the present study, association of obesity with raised MDA level was also found in T2DM patients. Das *et al*. also found that significantly increased MDA levels in obese diabetics than non-obese individuals. This study also proposes that oxidative stress may act as a connecting link between obesity and T2DM.[30] In certain studies along with serum MDA level, serum leptin level was also found to be increased in obese diabetic individuals. Pandey *et al*. found that hyperleptinemia and oxidative stress are strongly associated with each other in obese diabetics.[31] In fact, the study conducted by Stefanović *et al*. observed that increased oxidative stress and hyperleptinemia, both consequences of obesity, may play a role in T2DM development.[32]

In our study, diet intake, particularly mixed type of diet, was also found to be associated with raised serum MDA level in T2DM patients. In a study conducted by Carvalho *et al.* observed that, there was a positive association between increased level of serum MDA (oxidative stress) and high heterocyclic amine intake (in meat eaters) than vegetarian food intake. Thus our finding suggests that meat eating in diabetics may have an additive effect in development of oxidative stress.[33] Thus, it is suggested that to assess oxidative stress in T2DM and its role in the development of diabetic complications; serum MDA level should be assessed, especially in patients with risk factors such as obesity, long duration of diabetes, and mixed type of diet intake.

Limitation

The main limitation of our study is that it is a cross-sectional study, in which we could not establish causal relationship. To establish it, longitudinal study should be undertaken.

Strength

This cross-sectional study could be a good basis for future full-scale studies with a larger sample size. This study can help us to identify the oxidative stress level in T2DM patients and to take early measures so that we can prevent the progression of disease toward complications.

CONCLUSION

Serum levels of MDA were significantly increased in T2DM. It may be a good marker of oxidative stress that is implicated in various pathological conditions of T2DM. Elevated MDA levels are also associated with conventional risk factors such as diet, truncal obesity, and duration of diabetes.

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