Assessment of Range of Malonyldialdehyde in Serum and Some Anti-Oxidant Enzymes in Patients with Diabetes Type 2 with Controlled Blood Sugar In Comparison with the Diabetics with Uncontrolled Bs

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ABSTRACT

Diabetes disease and chronic high BS cause an increase in free radicals. Stress oxidative has an important role on the effects of diabetes in short term and long term. This study assess some oxidant and anti-oxidant markers in diabetics patients with controlled and uncontrolled blood sugar. Methods: 127 patients with diabetes type Z using B.S. reducing medicines (67 patients with controlled diabetes and 63 patients with uncontrolled B.S. are the samples of this study. Clinical assessment contains height, weight, BML, systolic and diastolic blood pressure. Biochemistry assessment contains FBS, glycolized hemoglobin, Malonyldialdehyde is serum catalase and dismutase super anti-oxidant enzyme’s functions. To analyzing the data spss (11) and to compare the data of two groups t-test in used.Findings: in uncontrolled diabetic patients type 2, Malonyldialdehyde’s mean in serum (2/01± 0/88), was significantly upper than controlled diabetes patients (1/63± 1/01) (p = 0/03). In controlled diabetic patients catalase’s function mean (148/126 ± 42/50) was upper and dismutase super oxidase (1159/38 ± 244/31) was lower than controlled diabetes patients (catalase: 136/95 ± 39/85, dismutase super oxidant : 1171/69 ± 229/45), these differences are not statistically meaning full. Conclusion: Malonyldialdehyde in serum in uncontrolled diabetic patients, so uncontrolled diabetes type 2, and chronic high BS, lipid peroxidation increases. The function of catalase in response to free radicals increases, but may decrease as a effect of free radicals, like decrease of dismutase super oxide.

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INTRODUCTION

Diabetes mellitus or diabetes type 2, is one of the most common noncontagious disease and one of the endocrine dysfunctions.[1] diabetes type z is related to insufficient production and release of insulin of beta cells of pancreas or resistance of the peripheral cells to insulin.[2]. It is anticipated that in 2030 diabetes type 2 reached 366 million people. According to this anticipation diabetes type 2 from 4/1 in 2000 reached to 6/0 in 2030 [3]. In other words 1/10 of the people will have this problem especially in developing countries [4] in a national study (NSRFNCD = National survey of risk factor for non-communicable disease of Iran, in 2008 shows 7/7 of the adults (25-64 years old) had diabetes type 2, and 16/8 have glucose [5].

Diabetes type 1, a chronic disease in which BS increase and some dysfunctions in metabolism of lipids, proteins and carbohydrates happens [2]. Diabetes type 2, causes micro and macro endothelial changes which are the main cause of the short term and long term effects of diabetes in liver, cardiovascular diseases, neuropathy, nephropathy, blindness and …. These diseases make life shorter and increase the health case [6,7]. Studies show that cardio vascular disease is one of the main effects of diabetes type 2 [8]. Increased blood sugar cause auto-oxidation of glucose, glycolization of protein without enzymes and activation of first method and these functions make stress oxidative [9]. Stress oxidative is caused by unbalanced production of free radicals or pre-oxidation and immune system of body which increase production of ROS (Reactive oxygen spices) conditions there is a balance between ROS and RNS. Stress oxidative is a condition in which production of RNS / ROS is higher than anti-oxidant capacity, and cause macro injury to cells, peroxidation of cell lipids, misshaping of proteins, DNA [11]. Stress oxidation cause injury to beta cells of pancreas and production and release of the insulin and reducing those cells [12] anti-oxidants are both endosion and exosion, some are enzymes and some

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are not. Catalase, super oxide dismutase, glutathione reductase, glutathione peroxidase, tyrosine reductase, paracsonase … are enzymes, vitamins A,C,E, carotenoids, glutathione, flavonoids, Q 10 co enzyme, zinc, copper, selenium … are not enzyme antioxidant [13] SOD is an enzyme CAT are two enzymes which change H₂O₂ to H₂O; these two enzymes are markers of cardio vascular injury in diabetes type 2, [14]. MDA (malonyl dialdehyde) is one of the last productions of lipid peroxidation and is indicator of stress oxidative in vitro PA increases in diabetic patients with macro angiopathy and micro albuminuria,[15]. Some study made related to lipid peroxidation and functions of anti oxidant enzymes in patients with diabetes type 2, which reported different report [16-21]. Stress oxidative caused by hyperglycemia is happened before bad effects of diabetes.

By controlling diabetes what will happen for anti-oxidant in the body? This study will assess the functions of anti oxidant enzyme, catalase and superoxide dismutase and MDA in serum of controlled and uncontrolled diabetic patients. Methods: this study is done on 127 patients with diabetes 2 which were chosen by convenient sampling from patients of Persian diabetes clinic in khorasan Razavi. The patients of this study have FBS upper than 126 mg/dl and hemoglobine (glicolizied)(H6AIC) ≥ 6, using blood sugar reducing medicenes, atleast 3 years having diabetes, at least 40 years old and satisfaction to being in test. Patients use insulin, having liver, kidney, thyroid and parathyroid problems, pregnant women, women give breast feeding, using anti blood pressure drugs, vitamins A,C,E can not participate in test. 63 patients have controlled and 64 patients have uncontrolled diabetes. Patients (H6A1C < 8), BMI assessment is done by seca assessed (seca 725 GM6H & co, Germany) patients were assessed without shoes and minimum clothes on the scale. Accuracy of the scale were 100 gr, and 0/5 cm. BMI is acquired by weigh (kg) on multiplied height, FBS was taken from patients 7-11 morning, after 12-14 hours fasting in 10cc blood from right hand’s vein, patients were sitting and blood is taken before BS reducing medicine, 5cc blood in first tube with 0/3cc EDTA as anticoagulation and 5cc in second tube without anticoagulation. Tube with EDTA were locked with Para film. To preparing serum and plasma, blood were centrifuged by circulating 1500g 10 minutes. Serum is taken from above of the tube blood without anticoagulation and plasma is taken from above of tube with anticoagulation. After separation of plasma, homoloyzed (sediment globules) were washed 3 times by physiologic serum 9/0% and were centrifuged by 1500 circulation 10 minutes, the samples of serum, plasma and hemoloyzeds kept in code tubes of every patients until test in freezer -80’c. for assessment of FBS oxidase glucose test and for H6A1C immunotormido metric with latex is used. Assessment of dismutase super oxidase enzyme’s function : measurement of dismutase super oxidase enzyme function in red blood cell is done by randox, No: sp 125 kit. In this method xezantin and oxide xezantin and oxide xezantin are used to produce super oxide radicals. These radicals with phenyl tetrazolim chloride make red formazoon complex which measured by light absorption with 505 nanometer wave length. If there is any enzyme, super oxid radicals change to H₂O₂ and O₂ and prevent making formazoon. Function of sod enzyme by preventing mentioned reaction and light absorption of formazoon complex in 505 nanometer light wave is measured.[22]

Measurement of catalase enzyme function : separation of peroxide H₂ by reducing light absorption in ultra violet is connected. By this character is assessed by spectro photo metry in 240 n.m. and differences in absorption 240 A Δ in time (30 seconds) is essential in measurement. To prevent inactivation of enzyme (usually in 30 seconds) or air bulb making in kut, it is necessary to use minimum rang (MM 10) H₂O₂. Dependence of enzyme’s function is weak temperature and can be measured in 0 - 37’c, but 20’c is recommended [23].

Measurement of MDA : MDA in serum is measured by spectro – photometry and tiobarbitoric acid. In this method MDA is measured by high sensitivity. Tiobar bitoric acid (TBA) in sodium sulphat is added to sample after giving heat, cromosion is achieved by butyl alcohol and the measure of light absorption in 530 n.m. of light wave is read. By using mentioned values, standard solution, MDA measurement is measured as nmol/ml.

Systolic and diastolic BP measurement: the patients rest 5 minutes, then blood pressure is measured by cuff and he cuff fasten 2/5 cm above elbow and recorded in standard statistical analyses : the results are shown by mean ± in tables. Statistical analyse is done by pair T-tests for mean of quantity values in controlled and uncontrolled diabetes patients and independent T-test is used for value grouping and comparison of them with independent values. To assessment of values, Pierson, and to analyze the data, spss 11 are used. This study is accepted in moral committee of Tehran medical sciences university and also confirmed. P-values < 0/05 is recognized as significantly.

Findings: In this study 127 patients with diabetes type 2, using BS reducing medicenes were participate. According to H6A1C, 64 patients is defined controlled diabetic and 63 patients, uncontrolled, 9 patients of the participating patients were female (58/7%) and 48 patients were male (41/3%). Demographic data were age, gender, BMI, diabetes duration, systolic and diastolic B.P. in controlled and uncontrolled diabetes groups and shown in table 1.

According to data of this table, age of the patients in controlled diabetes were significantly upper than patients with uncontrolled diabetes were significantly upper than patients with uncontrolled diabetes (P=0/03). Other values of this table have no significant differences. Table 2 show biochemistry data such as MDA and anti
oxidant enzyme (catalase and dismutase super oxidase in both groups. MDA in patients with controlled diabetes was lower than uncontrolled diabetes patients (P<0.03), the difference is statistically significant. But the function of the mentioned enzyme is not statistically meaningful. Table 3 show age, BMI, duration of disease FBS, systolic and diastolic blood pressure and their relations with MDA and catalase and super oxide dismutase in both groups. FBS, systolic and diastolic BP have meaningful relation with MDA. age and catalase enzyme have adverse and meaningful relation. Table 1 show demographic and anthropometric data in both groups. Sample is 64 controlled diabetes patients and 63 uncontrolled diabetes, data is shown as mean ± SD, P < 0.05.

Table 2 show biochemistry data, mean ± SD MDA, catalase, superoxide dismutase, MDA : malonyldiadehid, sob ; super oxide dismutase, CAT : catalase, statistical method is independent T-test, P < 0/05

Discussion: according to data and results of the study MDA in serum of controlled diabetes 6<H6A1C ≤ 8), controlled patients (H6A1C < 8) was significantly meaningful. Peerapatdit & co. made a study for 3 diabetic groups in comparison with healthy people. Group 1: controlled diabetes FBS ≤ 180 mg/dl, group 2:uncontrolled FBS 180 mg/dl, groupe 3 : diabetics with cardiovascular disease. MDA in first two groupes specialy diabetics with cardiovascular disease was significantly upper than group 1. So by controlling blood sugar we can decrease MDA.[25]. This result harmonize with other studies [20,21,26,27]. There is a meaningful relation between uncontrolled diabetes, FBS and MDA.[28-30].Which is seen in our study. In stress oxidative condition free radicals cause lipids peroxidation of cell membrane [16]. So MDA is a good significant to show stress oxidative and other metabolic disease. On study show SOD in controlled diabetes patients is a little lower than uncontrolled diabetic patients. Other study shows SOD is high in the first stage of diabetes type 1 and 2, by controlling diabetes and insulin intake, it reduced but never reach normal stage [31]. In other study SOD in diabetics with cardiovascular disease and patients with weak controlled of diabetes was meaningfully upper than normal people. SOD in patients with diabetes mellitus in comparison with control group was meaningfully higher.[25]. Our study shows catalase in patients with high FBS was meaningfully upper than controlled group. Taher and co> in a study made on 100 patients with diabetes type 2, using blood sugar medicines, shows that in diabetics, super oxide dismutase was lower and function’s rate of glutamine peroxidase and glutamine redoctose was upper than healthy people [32,33]. In study made by peerapatdit and co. catalase in 3 groups (which is mentioned earlier) was upper than control group, according to age and gender [25].

About stress oxidative, pan and co in 2009 on patients with diabetes and nephropathy shows that MDA and conjugated diene (markers for lipid oxidation) protein carbonyl (pc), advanced oxidation protein products (protein oxidation markers) in comparison with diabetic patients without nephropathy was meaningfully upper.[34]. Adlibas in a study made in 2011 shows oHdG-8 as a determine for DNA destruction and MDA increase and the reta of SOD in diabetics with retinopathy in comparison with diabetics without retinopathy [35]. about antioxidant enzymes studies show controversy. SOD in some studies increase [27,36,38], reduced [34,39], or without changes [40,41]. OAT in some studies increase in some decrease and in some studies recorded without changes [16, 26, 40, 42, 43], pasaoegl and co, in a study made on 3 diabetics group about antioxidant. Patients with new diabetes, patients which use blood sugar reducing drugs and healthy people as controlled group.

The study shown that in early stages antioxidant system of the body fight free radicals but gradually become weak [28].

SOD and other antioxidant enzymes like other proteins in cells are attacked by ros and autoglycolization. In this situation proteins changed.

Studies show that 50% proteins in RBC of the patients with diabetes type 2 become glycolized [44]. KM of catalase is lower than superoxide so it is anticipated that in red blood cells SOD is weaker than catalase in facing with attack of glycolization.

Hamden and co. in a study done on rats with diabetes caused by Alloxan show that hyperglycemia cause reduction in cu+2 level which is an important co-factor for superoxide dismutase. Glycolisation SOD cu/zn in (uncontrolled) diabetic patients is seen and sod and SOD glycolisation in RBC inactivate this enzyme.

Changes of MDA and SOD in uncontrolled diabetic patients show hyperglycemia cause stress oxidative which is made by peroxidation of lipids.[33]

So this study and other studies show that diabetes type 2 increase MDA level which is caused by lipid peroxidation as a response to hyperglycemia and continue of hyperglycemia reduce the function of the enzyme which is caused by ROS.

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