Study of Correlation between Malonyldialdehyde Levels and Resting Heart Rate in Depressive Subjects

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Abstract: Background: There are several molecular changes in the pathogenesis of depression. The aim of our study was to correlate Malonyldialdehyde Levels and Resting Heart Rate in male depressive subjects. Material and Methods: The present observational case control study was conducted in Department of Physiology for a period of three years. A total of 83 depressive cases diagnosed by Psychiatrist were included in the study. Additionally a total of 100 normal individuals without any psychiatric disorder were taken as controls. Non-probability purposive sampling method was adopted for selection of subjects. Correlation between antioxidant malonyldialdehyde levels and heart rate was studied. Results: There was statistical significant correlation observed between malonyldialdehyde with resting heart rate of control group (Unpaired't' test, p value < 0.05) Conclusion: The present study concludes that utility of Malonyldialdehyde Levels and resting heart rate as autonomic function test parameter plays an important role in monitoring management of depressive patients. [Shashikant S Natl J Integr Res Med, 2019; 10(3):34-39]

Key Words: : Malonyldialdehyde , resting heart rate , depressive patients

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Introduction: Disturbances in the antioxidant defense mechanism can play a part in a wide range of neuropsychiatric disorders.1 The antioxidant defense mechanisms protect the cells by removing the free radicals. Excessive lipid hydroperoxides overpower the antioxidant system to form lipid peroxidation products such as 4 - hydroxynonenal, malonyldialdehyde or 8- isoprostane. Lipid peroxides not only cause oxidative stress but influence lipid signaling, monoamine regulation, inflammatory pathways and autoimmune activity through formation of immunogenic neo-epitopes causing pathophysiology of major depressive disorder (MDD).[2] Even a slight change in antioxidant defense mechanisms and oxidative stress can be deleterious to neurons. The molecular mechanism related to oxidative stress represents one of the factors in the etiology of depression and anxiety disorders.[3] onsidering the association of autonomic dysfunction with its effect on morbidity and mortality this study was carried out to assess resting heart rate in depression and compare them with control group. Also because depression is associated with oxidative stress it was decided to study levels of malonyldialdehyde in depression and compare them with normal controls.

Material and Methods: The present observational case control study was conducted in the Department of Physiology in collaboration with Department of Psychiatry, Rural Medical College and Hospital, Pravara Institute of Medical Science (DU).The study protocol was approved by Institutional Ethics Committee, Loni, for the period of three years . Signed informed consent was obtained from all participants. The Non-probability purposive sampling method was used for those satisfying the inclusion criteria.

For selection of control (Group I) the inclusion criteria was the controls were free from depression with males in the age group 20-55 years and they were willing to participate in the study whereas the exclusion criteria was no history of consumption of psychotropic substances and patients suffering from other co-morbidities (HTN, COPD, Asthma, Diabetes) were excluded from the study.

For selection of depression cases (Group II) the inclusion criteria was the depression cases were diagnosed by psychiatrist & rated on the basis of Hamilton's depression scale with males in the age group 20-55 years and they were willing to participate in the study whereas the exclusion criteria was history of consumption of psychotropic substances and patients suffering from other co-morbidities (HTN, COPD, Asthma, Diabetes) were excluded from the study. First a written consent was obtained from all subjects. The blood sample was collected in plain bulb from each case & control from cubital vein with all aseptic precautions. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Antioxidants test conducted in cases and controls using Buege and Aust 1978 [20] , which has normal

value of Serum Malonyldialdehyde (MDA) 2 to 2.5 nmoles /milliliter

"CANWIN" Cardiac Autonomic Neuropathy Analyzer: Cardiac Autonomic Neuropathy Analyzer was used to assess resting heart rate. It is a fully automatic windows based instrument which gives graphical interpretation and keeps subject's data. As it is automatic, recordings, readings and calculation becomes possible.

Autonomic function test in cases and controls: Resting heart rate : Methods to Estimate serum malonyldialdehyde (MDA) Method: Buege J and Aust S 1978 Microsomal lipid peroxidation. Methods in enzymology (105), 302-3104. Principle: Malonyldialdehyde (MDA) is highly reactive three carbon dialdehyde, produced from lipid hydroperoxidation. It can, however, also be derived from hydrolysis of pentoses, deoxyriboses, hexoses, from some amino acids and form DNA. MDA estimation was done by thiobarbituric acid reaction. MDA is measured as an index of lipid peroxidation.

Procedure: - Serum sample was first treated with trichloroacetic acid (TCA) for protein precipitation and then treated with thiobarbituric acid (TBA). The mixture is heated for 10 minutes in boiling water bath one molecule of MDA react with two molecules of thiobarbituric acid. The resulting chromogen is centrifuged and intensity of colour developed in supernatant is measured colorimetrically at 530 nm. MDA levels are expressed in nmol/ml.

Reagents: 40 % TCA (Trichloroacetic acid):40 gm of TCA in 100 ml of distilled Water. 0.67 % TBA (Thiobarbituric acid): dissolve 0.670 gm of TBA in 100 ml of distilled water in boiling water bath.

Standard malondialdehyde (MDA) .The stock MDA was prepared from the 1, 1, 3,3-tetraethoxy propane by acid hydrolysis. A solution containing 5 µl 1,1,3,3- tetraethoxy propane in 50 ml of distilled water and 0.1 ml of 0.1M HCL is warmed at 50 degree centigrade for 1 hour and volume adjusted to 100 ml with distilled water. The concentration of free MDA was determined spectrophotometrically at 267 nm, using a molar coefficient of 31,800.

MDA standardization : Prepare working standard of MDA by taking 1 ml of stock and diluting it up to 100 ml with distilled water. Now with this working standard prepare various dilutions and make additions as follows.(Table-1)

Table 1: MDA Standardization

Sr. No	Std no	Std (ml)	DW (ml)	Total volume	Conc. of std. (nmol/ml)
B	B	0.0	1.0	1.0	-----
1	S1	0.1	0.9	1.0	2.22
2	S2	0.2	0.8	1.0	4.44
3	S3	0.3	0.7	1.0	6.66
4	S4	0.4	0.6	1.0	8.88
5	S5	0.5	0.5	1.0	11.1
6	S6	0.6	0.4	1.0	13.32
7	S7	0.7	0.3	1.0	15.54
8	S8	0.8	0.2	1.0	17.76
9	S9	0.9	0.1	1.0	19.98
10	S10	1.0	0.0	1.0	22.20

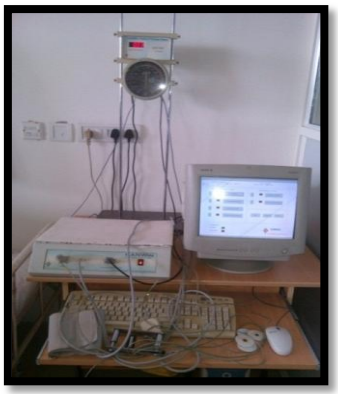
Then to each test tube add 1 ml of 40 % TCA, mix well. Add 2ml of 0.67 % TBA. The coupling of lipid peroxide and TBA was carried out by heating in water bath for 10 minutes.The resulting chromogen was cooled and its absorbance against MDA standard concentration was plotted(Table-2).

Table 2 :Sample processing

NO	REAGENTS	QUANTITY
1	Serum	1.0 ml
2	40% TCA	1.0 ml
3	0.67 TBA	1.0 ml

The above reaction mixture was heated in boiling water bath for 10 minutes. It was then cooled at room temperature and centrifuged. The absorbance of supernatant at 530 nm was noted. Resting Heart rate was carried out with the help of CANWIN" Cardiac Autonomic Neuropathy Analyzer(Fig. 1).

Figure 1: CANWIN" Cardiac Autonomic Neuropathy Analyzer in Department of Physiology



Results: In the present study as shown in table 1.1, there was no statistical significance observed in age and height of control and depression cases, whereas there was a significant difference between the mean values of weight in category II and category III of control and depression cases (Unpaired't' test, p value < 0.05)

Table 3: Comparison of anthropometric parameters in group I and group II

Anthropometric parameter	Control (Group I) n = 100 Mean± SD	Depression cases (Group II) n = 83 Mean ± SD		P value
Age (years)				
Category I 20-30 years (n=26)	26.35 ± 2.53	Category I 20-30 years(n = 11)	26.45 ± 2.68	0.05
Category II 31-40 years (n = 38)	35.75 ± 2.80	Category II 31-40 years(n = 39)	36.03 ± 3.10	0.05
Category III 41-55 years (n = 36)	46.72 ± 4.14	Category III 41-55 years(n = 33)	46.91 ± 3.48	0.05
Height (Cms)				
Category I (26)	154.35 ± 4.60	Category I (11)	155.55 ± 8.37	0.05
Category II (38)	156.71 ± 4.83	Category II (39)	152.54 ± 6.90	0.05
Category III (36)	155.33 ± 5.30	Category III (33)	152.42 ± 7.33	0.05
Weight (Kg)				
Category I (26)	51.98 ± 8.55	Category I (11)	51.00 ± 7.33	0.05
Category II (38)	54.11 ± 8.52	Category II (39)	50.62 ± 6.31	0.04
Category III (36)	53.83 ± 7.47	Category III (33)	48.15 ± 6.10	0.03

Table 4: Comparison of malonyldialdehyde in group I and group II

Antioxidants	Control (Group I) n=100 Mean ± SD	Range	Depression cases (Group II) n = 83 Mean ± SD		Range	P value
Level of oxidative stress						
Malonyldialdehyde (MDA) (nmol/ml)						
Category I (26)	2.02 ±0.16	1.84-2.45	Category I (11)	6.28 ±0.7	5.01-7.12	0.05
Category II (38)	2.01±0.16	1.84-2.45	Category II (39)	5.31 ±1.3	3.11-7.23	0.04
Category III (36)	2.02±0.15	4.0-7.23	Category III (33)	5.74 ±1.1	4.0-7.23	0.01

There was statistical significant correlation observed between malonyldialdehyde with resting heart rate of control group (Unpaired't' test, p value < 0.05)

Table 5 : Level of Oxidative Stress

Malonyldialdehyde MDA	0.0 (0.49)	Malonyldialdehyde MDA	- 0.0 (0.78)
*Note: r = Correlation coefficient, Value in bracket indicate= *p* value (Unpaired't' test, *p* value < 0.05)			

In table 6, the parasympathetic functions such as resting heart rate/min are shown.The resting heart rate/min was significantly increased in depression cases as compared to control (Unpaired't' test, *p* value < 0.05).

Table 6: Comparison of resting heart rate/min in group I and group II

Parasympathetic functions	Control (Group I) n=100 Mean ± SD	Range	Depression cases (Group II) n = 83 Mean ± SD		Range	P value
Resting heart rate/min						
Category I (26)	76.15 ±5.84	65–88	Category I (11)	81.09 ±6.36	72–90	0.00
Category II (38)	73.58 ±6.24	62–83	Category II (39)	80.59 ±6.87	63–94	0.04
Category III (36)	76.97 ±7.51	60-96	Category III (33)	80.55 ±8.23	60–96	0.03

Table 7: Correlation between malonyldialdehyde and resting heart rate in group I and group II

	Control (Group I)	Depression cases (Group II)

Sr. no	Type of Antioxidant	r (p)*	Type of Antioxidant	r (p)*
1	Malonyldialdehyde MDA	0.0 (0.49)	Malonyldialdehyde MDA	- 0.0 (0.78)
*Note: r = Correlation coefficient, Value in bracket indicate= p value (Unpaired‘t’ test, p value < 0.05)				

Discussion: Studies have reported that there is a definite autonomic imbalance in patients suffering from depression. On this basis, in the present study, levels of Malonyldialdehyde and resting heart rate in patients of depression were compared with healthy normal controls. In total, 183 males were included in the present study consisting of 100 healthy normal controls and 83 patients suffering from depression. They belong to age group 20-55 years and were further subdivided into category I (20-30 years), category II (31 – 40 years) and category III (41 -55 years). In the present study only male patients were included because female patients not supportive and denied to participate.

In the present study the antioxidant enzymes estimated is malonyldialdehyde (MDA) which was measured as a biomarker of the oxidative stress. Malonyldialdehyde (MDA) is a biomarker widely used by researchers so as to verify the damage caused by lipid peroxidation, therefore gives an idea regarding the oxidative stress status. In the present study, there was a significant difference observed in mean values of malonyldialdehyde levels in control (group I) as compared to group II which consists of patients suffering from depression. (Unpaired ‘t’ test, p value <0.05) (Table No.1.2) In the present study significant elevation in the mean values of MDA Levels in depression was found. Similar, such results were observed by other research workers.5, 6, and 7. D'Souza B et al (2003) found significant increase in oxidative stress and lipid peroxidation in schizophrenia.8 Similarly, Rukmini M, et al (2004) in her study also found significantly higher levels of malonyldialdehyde in patients suffering from depression as compared to the control subjects. This makes it further evident that there is increased oxidative stress in depression.[5] Bajpai A et al (2014) suggested that prolonged psychological stress is the etiology for major depression which leads to increase in malonyldialdehyde levels, oxidative stress and triggers the depressive symptoms in the patient’s affected.[6]Liu T et al (2015), in their meta-analysis study on oxidative stress markers in depression found significantly higher levels of malonyldialdehyde.[7] Mazereeuw G et al (2015), in their study reported an association between the lipid peroxidation studied in peripheral blood samples and major depressive disorder (MDD) symptoms. This finding emphasizes the fact that with a general increase in oxidative stress in the presence of symptoms of depression there is specific elevation of lipid peroxidation. There was a linear relationship suggested between the increased severity of depression symptoms and greater degree of lipid peroxidation in patients with major depressive disorder (MDD).[9] Atmaca M et al (2004), reported significant elevation in the levels of malonyldialdehyde in social phobia. In their study, malonyldialdehyde levels were lowered after eight weeks of treatment with citalopram.10 Kuloglu M et al (2002) found significant positive correlation between Panic Agoraphobia Scale (PAS) score and malonyldialdehyde levels.[11] Dadheech G et al (2006), in their study reported significantly elevated malonyldialdehyde levels as observed in the present study. The raised malonyldialdehyde is indicative of oxidative injury due to schizophrenia. The injury is a resultant of free radical formation and chain reaction of damage initiated by extracting hydrogen atoms from lipoproteins and lipids of bio-membranes causing lipid peroxidation, of which malonyldialdehyde is the main product. Similar studies by the same authors in 2008 confirmed that increased levels of malonyldialdehyde existed in 50 patients of schizophrenia studied and indicated the existence of oxidative stress in schizophrenia.[12]

Can M et al (2011) in his study reported seven folds increase in MDA levels.[13] The results indicate a statistical significance correlation as observed between antioxidants like malonyldialdehyde and resting heart rate (RHR) in control subjects (group I). Similar results are obtained between malonyldialdehyde and resting heart rate in control subjects (n =100) (Unpaired‘t’ test p value <0.05) (Table no. 5)

The study of heart rate is important to assess the parasympathetic functions of the body. Researchers have studied on different types of heart rates i.e., resting heart rate, heart rate variability to assess the parasympathetic functions of patients suffering from depression. In the present study we found significant increase in resting heart rate in depression. Similar results were observed by other studies. Carney R, et al (1999) suggested that depression is associated with altered autonomic activity.[14] Alvares G et al (2016) in their study reported reduced heart rate variability in patients suffering from psychiatric disorders. Further, they also studied effect of psychotropic medication on heart rate variability (HRV). They observed that ANS dysfunction is a characteristic feature in otherwise healthy patients with major psychiatric disorder, with the largest effects observed in patients with psychotic disorders and psychotropic medications.[15]

Ehrenthal J et al (2010), in their study compared cardiovascular reactivity to anger recall and mental arithmetic tasks in 25 patients with severe depression without heart disease and 25 non-depressed subjects. The depressed patients exhibited overall reductions in heart rate, blood pressure and cardiac index.[16] Jahan C et al (2014), found decreased heart rate variability ($p< 0.001$) in depressive patients as compared to healthy controls. The authors suggested lower parasympathetic and higher sympathetic drive as well as higher low to high frequency (LH/HF) ratio as an index of sympathovagal imbalance in major depressive disorder (MDD) patients.[17] Wang Y et al (2013) more recently suggested that depression is associated with dysfunction of the cardiac autonomic nervous system; also the severity of depression is directly proportional to the severity of this dysfunction. It appears that patients with depression are susceptible to premature atrial and/or ventricular disease.[18]

By studying and measuring autonomic function such as heart rate the role of parasympathetic nervous system in the physiology of depressive patients is known. Thus present study gives a clear idea regarding status of antioxidant defense system, level of oxidative stress status and the role of autonomic nervous system, in patients suffering from depression as compared to healthy controls.

Conclusion: The present study concludes that utility of Malonyldialdehyde Levels and resting heart rate as autonomic function test parameter plays an important role in diagnosis, treatment and monitoring of depressive patients.

Limitations: Herewith in present study we were restricted to only two parameters viz. malonyldialdehyde Levels and resting heart rate as autonomic function test basic parameter. Follow up study of malonyldialdehyde and resting heart rate was not performed. As all the cases in the study and control group are males, findings of this study may not be fully indicative of similar changes in females.

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